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Effects of menstrual cycle phase or
monophasic oral contraceptive use on body
temperature, mood and somatic symptoms,
and utility of different self-monitoring
methods

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A dissertation submitted in partial fulfilment of the
requirements for the degree of Masters (by research and
thesis) in Sport Science

School of Sport and Exercise Sciences

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July 2021

Abstract: More research is needed to investigate the hormonal differences and the effects they may have on females, with or without oral contraceptive use, in order to help increase our understanding of female physiology and to help create female specific guidelines for research and practical settings. **PURPOSE:** To investigate if the menstrual cycle or monophasic oral contraceptive (OC) use influences body temperature, mood or somatic symptoms and to assess menstrual cycle self-monitoring methods in at home settings. **METHOD:** 4 eumenorrheic females and 6 monophasic OC users tracked their menstrual cycle over 2-3 months using basal body temperature (BBT), urinary luteinising hormone detection kits and self-report logs. They also completed several questionnaires throughout each cycle. **RESULTS:** *Non-OC users:* we failed to find statistically significant differences across the menstrual cycle in eumenorrheic females. Analysis revealed a main effect of menstrual cycle phase for pain, although post-hoc analysis did not identify any specific phase difference. A large effect size (Cohen's $d = 0.88$) was however observed for the difference between means of premenstrual and early follicular phases (Mean \pm standard deviation (SD): premenstrual 9.5 ± 3.2 vs early follicular 12.7 ± 4.4 ; participants scored their results on a 6-point Likert scale according to severity of experience, with higher values indicating greater severity). *OC users:* unsurprisingly, we failed to find significant differences for the majority of parameters measured for OC users. There was a significant difference found between means of behavioural change between the start and end of withdrawal week (Mean \pm SD: start of withdrawal week 6.8 ± 2.0 vs. end of withdrawal week 6.3 ± 2.0 , $P = 0.001$; scores recorded on a 6-point Likert scale according to severity of experience, with higher values indicating greater severity). **CONCLUSION:** The results indicate that body temperature, mood and somatic symptoms were not different across the menstrual cycle for eumenorrheic females. Similar results were seen in monophasic oral contraceptive users. At home luteinizing hormone detection kits and basal body temperature recordings were simple and easy to carry out at home. The BBT recordings, however, were not successful in displaying temperature changes throughout the menstrual cycle in eumenorrheic females.

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I would also like to thank the participants for their invaluable contribution to the study.

Declaration

“No part of this thesis has been submitted in support of an application for any degree or other qualification of the University of Kent, or any other University of Institution of learning.”

Signed: Gabriella Michaela Swart

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Definitions

Abbreviation

HC

OC

FSH

LH

ER

ER α

ER β

PR α

PR β

MVIC

MVC

VO_{2max}

CK

IL-6

BBT

°C

mRNA

PMS

Non-OC

OC users

MDQ

CNS

EE

SHBG

fMRI

ANOVA

EDO

PRISM

STAI

SCL

COVID-19

Meaning

Hormonal contraceptive

Oral contraceptive

Follicle-stimulating hormone

Luteinizing hormone

Oestrogen receptor

Oestrogen receptor alpha

Oestrogen receptor beta

Progesterone receptor alpha

Progesterone receptor beta

Maximal voluntary isometric contraction

Maximal voluntary contraction

Maximum rate of oxygen consumption

Creatine kinase

Interleukin-6

Basal body temperature

Degrees Celsius

Messenger ribonucleic acid

Premenstrual syndrome

Eumenorrheic females

Oral contraceptive users

Menstrual distress questionnaire

Central nervous system

Ethinyl estradiol

Sex-hormone binding globulin

Functional magnetic resonance imaging

Analysis of variance

Estimated day of ovulation

Prospective record of the impact and severity
of menstrual symptoms

State trait anxiety inventory

Symptom distress checklist

Coronavirus disease 19

I. Introduction

Over the last few years, the number of females participating in sports has increased dramatically (Fink, 2015), however sports participation inequality between the two sexes remains (Deaner et al., 2012; van Tuyckom, Scheerder, & Bracke, 2010). The inequality is also seen in performance-based research (Costello, Bieuzen, & Bleakley, 2014; Mujika & Taipale, 2019). Between 2011 and 2013, only 39% of studies published in the journal *Medicine and Science in Sports and Exercise* included research on females. Since then, there has been an increase in female inclusion in research, however, it is still unequal (Bruinvels et al., 2017; Costello et al., 2014; Sheel, 2016). When looking at the first 5 issues of the *Journal of Sports Physiology and Performance*, published in 2019, only 19% of studies included females, with only 4% exclusively focusing on females. Moreover, the majority of the literature that explored females in sport performance has often disregarded the menstrual cycle and how the hormone levels may affect performance (Del Coso et al., 2013; Johnson, Greaves, & Repta, 2009; Lara et al., 2014), which makes it challenging to apply findings on this to females in sport (Emmonds, Heyward, & Jones, 2019). In addition to the above, tracking the menstrual cycle is vital to determine which menstrual phase the females are in and how it may affect sporting performance. It should be noted however that even in eumenorrheic females regular hormone concentration fluctuations cannot be assumed and therefore testing is vital to help validate the hormones (Scheid & De Souza, 2010).

Hormonal contraceptive (HC) use within the athletic population is common, with approximately 50% of elite athletes currently using it (Martin, Sale, Cooper, & Elliott-Sale, 2018), yet, the majority of research does not take this into account. Many studies have grouped naturally cycling females and oral contraceptive (OC) users in the same category, which is not appropriate due to the differences in hormonal profile. The lack of research and consideration for gender and hormonal differences between sexes leads to generalization and inaccurate findings. Moreover, due to the underrepresentation within the literature, male data is often applied to females, even though there are anatomical, physiological and endocrinological differences between the sexes (Heidari, Babor, De Castro, Tort, & Curno, 2016; Pitchers & Elliot-Sale, 2019). More studies are therefore needed to investigate the hormonal differences in order to help increase our understanding of female physiology,

including the menstrual cycle and/or HC (Nimphius, 2019) and help create female specific guidelines for research and practical settings.

This project aims to determine whether the menstrual cycle or oral contraceptive use influence basal body temperature, mood and somatic symptoms. It also aims to determine the utility of self-monitoring methods. The first section of the literature review includes an overview of the menstrual cycle, an explanation of the hormone levels throughout the menstrual cycle and their effects within the body with relevance to the topic at hand. The thesis goes on to consider how the menstrual cycle influences muscle function and thermoregulation. The second section of the literature review explores the menstrual cycle and psychological effects, with an overview of physical and psychological symptoms associated with the menstrual cycle. The thesis then explores the affective and somatic symptoms and how they may be associated with the menstrual cycle at a greater depth. The eumenorrheic literature review concludes with an exploration into how the menstrual cycle may influence rewards and motivation. The third part of the literature review includes an overview of hormonal contraceptives and explores the synthetic hormones and how they may influence the body, including an exploration into how monophasic oral contraceptives influence thermoregulation. The fourth part of the literature review examines oral contraceptive use and psychological effects, including rewards and motivation, with a similar structure to section two of the literature review. Section five of the literature review reviews methods used for validating menstrual cycle phase, which includes an overview of self-report, BBT and urinary luteinizing hormone (LH) measurements. The final section of the literature review contains the aims of the research study.

Impact of Coronavirus disease 19 (COVID-19) and approach taken in this thesis

The original thesis title was ‘The acute effects of caffeine on short duration, high intensity performance with specific focus on the menstrual cycle phases’. Approval for this project was granted early in 2020 and data collection for this study and a pilot study was about to commence when the first national lockdown in the UK was announced (see appendix A for criteria and methodology). The data collection, and study, was put on pause initially, as at the time, we believed that the lockdown would be in place for a relatively short period of time. However, when it became clear that laboratory and routine data collection would not be possible in the near future, the project was changed to an at home-based study, which would allow data collection in a home setting. The change in study required considerable time to plan the project, recruit participants and distribute the materials to participants. Approval for the project was granted in July 2020 and thus data collection started.

II. Review of literature

The menstrual cycle

Overview

The menstrual cycle is defined as the cyclical changes of the ovaries and the lining of the uterus which repeats monthly, beginning at puberty and ending at menopause (Thiyagarajan & Jeanmonod, 2019). The menstrual cycle is regulated by changes in specific hormones; the gonadotrophin-releasing hormones, follicle-stimulating hormone (FSH) and LH, and the sex steroid hormones (oestrogen and progesterone) (Boivin & Shechter, 2010; Messinis, Messini, & Dafopoulos, 2014) (figure 1). The cycle can be divided into two distinct main phases, the follicular phase and the luteal phase, which can be further spilt into subphases. The subphases help highlight any changes that may occur due to the hormonal fluctuations in each main phase.

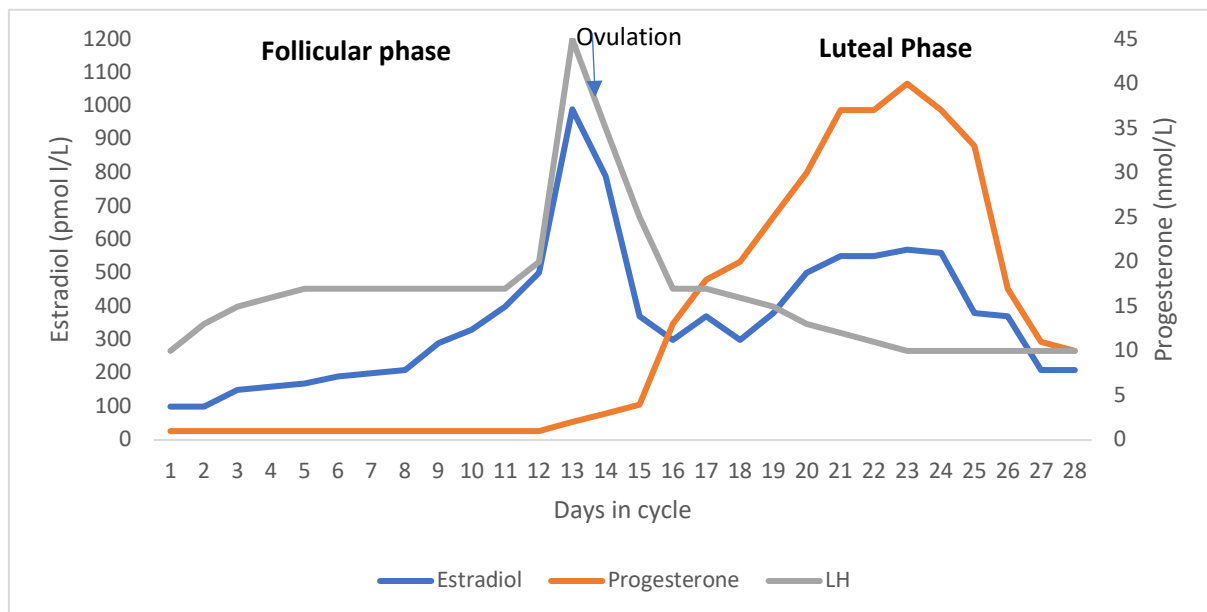


Figure 1 Estradiol and progesterone over a typical '28 day' menstrual cycle

The follicular phase begins from the first day of menstrual bleeding until ovulation (~ 12 - 14 days in a 'typical' cycle) (Bull et al., 2019). The early follicular phase consists of low oestrogen and progesterone levels, whereas in the late follicular phase oestrogen levels begins

to rise, due to FSH stimulating growth and development of ovarian follicles, and progesterone levels remain low (De Jonge, Thompson, & Han, 2019). The increase in oestrogen levels cause a surge in LH, released from the pituitary gland, which initiates ovulation. It should be noted that individuals may not ovulate every menstrual cycle (Fairley & Taylor, 2003), if ovulation does not occur the cycle is termed an anovulatory cycle. The luteal phase starts after ovulation and continues to the onset of menses; this phase consists of high oestrogen and progesterone levels (figure 1). It is worth mentioning, however, that oestrogen levels are not as high in the luteal phase as they are in the preovulatory phase.

The length of the menstrual cycle is the number of days from the first day of menstrual bleeding of one cycle to the day before the subsequent bleeding onset of the next cycle. A typical menstrual cycle lasts 28 – 30 days (Bull et al., 2019; Faust et al., 2019; Harlow, Lin, & Ho, 2000; Rosner & Sarao, 2019). In a typical cycle, the luteal phase is relatively consistent between women (~14 days) (Reed & Carr, 2000), whereas there is greater inter individual variance in the follicular phase, which usually vary between 10 - 16 days (Bull et al., 2019; Cole, Ladner, & Byrn, 2009; Faust et al., 2019; Fehring, Schneider, & Raviele, 2006; Frankovich & Lebrun, 2000; Mihm, Gangooly, & Muttukrishna, 2011; Mumford et al., 2012; Sherman & Korenman, 1975). Females who experience a typical menstrual cycle length are termed eumenorrheic. Females who experience menstrual cycle lengths less than 21 days are termed polymenorrhagia, and individuals who experience longer menstrual cycles (> 35 days) are termed oligomenorrheic.

Key hormones: oestrogen & progesterone

Oestrogen and progesterone are steroid hormones, secreted primarily from the ovaries. Oestrogen binds to oestrogen receptors (ER), including oestrogen receptor alpha (ER α) and oestrogen receptor beta (ER β) (Hansen, 2018; Ikeda, Horie-Inoue, & Inoue, 2019; Lee, Kim, & Choi, 2012). Progesterone binds to two receptor proteins: progesterone receptor alpha (PR α) and progesterone receptor beta (PR β) (Brinton et al., 2008; Spelsberg, Steggles, Chytil, & O'Malley, 1972). Although the primary role of both hormones is to support reproduction, they have been suggested to play a role in other physiological systems.

Muscle function

Several studies have demonstrated that menopause-related strength loss is connected to a decrease in oestrogen concentration, suggesting that oestrogen plays a role in muscle function (Samson et al., 2000). Skeletal muscle is the largest tissue containing oestrogen receptors (Wiik, Ekman, Johansson, Jansson, & Esbjörnsson, 2009), further suggesting that there may be a connection between the hormone and skeletal muscle. Collins et al. (2018) studied ER α deletion in mice and found that skeletal muscle contractility was impaired. Similar results were concluded by Cabelka et al. (2019) which helps signify that ER α is necessary for optimal muscle force production (Collins, Laakkonen, & Lowe, 2019; Lowe, Baltgalvis, & Greising, 2010). Moreover, oestrogen may also affect myofiber growth and regeneration. Kitajima and Ono (2016) studied mice with oestrogen reduction compared to mice with sufficient oestrogen. Mice with insufficient oestrogen had significantly compromised muscle regeneration compared to mice with sufficient oestrogen. The mice with insufficient oestrogen also had impaired satellite cell expansion, differentiation and self-renewal. The study highlighted that oestrogen was essential for maintaining muscle function, including muscle recovery. Similar findings are suggested by McClung, Davis, Wilson, Goldsmith, and Carson (2006) and Diel (2014). It would therefore be probable to expect that when oestrogen levels are high, muscle recovery and/or strength may be increased. Bambaiechi, Reilly, Cable, and Giacomoni (2009) studied sedentary females across 5 phases: 1-4 days from first day of menstrual bleeding, days 7-9 (mid-follicular), around ovulation (confirmed by urine LH spike), days 19-21 (mid-luteal) and days 25-27 (late luteal). They found significantly higher values for maximal voluntary isometric contraction (MVIC) of knee flexors around ovulation (62.7 N.m), when oestrogen levels are at their highest, compared to the mid-follicular phase (58.7 N.m) and mid-luteal phase (56.4 N.m). There was however no significant change in MVIC of knee extensors. Additionally, the study demonstrated differences in peak torque of knee flexors and extensors across the menstrual cycle, with an increase in peak torque after ovulation. Tenan, Hackney and Griffin (2016) investigated five phases. They found that maximal voluntary contraction (MVC) of the knee extensors decreased by 23% from ovulation to the mid luteal phase, but when individuals reached the late luteal phase, MVC returned to values similar to the other phases. This could be due to progesterone reaching its peak and therefore potentially having a negative effect on strength production. De Jonge, Boot, Thom, Ruell, and Thompson (2001) however, studied 19 healthy women with regular menstrual cycles. They assessed muscle strength, fatigability and

contractile properties of each participant in three phases: the early and late follicular phases and the luteal phase. The study concluded that there was no change over the menstrual cycle for any parameters studied. Similar results were concluded by Fridén, Hirschberg, & Saartok (2003). Montgomery & Shultz (2010) investigated recreationally active individuals. For a 2-month period prior to testing, individual's serum was obtained for 6 days after onset of menstrual bleeding (early follicular phase) and for 8 days after a positive LH test (luteal phase). The researchers investigated and tracked the individuals' estradiol, progesterone and testosterone concentrations in order predict and set dates for testing. Individuals were tested during the early follicular phase and luteal phase. During MVIC testing, serum hormone levels were also obtained. The study found no changes in MVIC of the knee flexors and extensors between the two phases. The discrepancies between the studies could be due to methodological factors (Pereira, Larson, & Bembien, 2020). For example, the number of experimental trials over the menstrual cycle; many of the studies only tested two – three phases within the menstrual cycle, whereas Bambiaichi et al. (2004) and Tenan et al. (2016) tested in five phases. Moreover, several of the studies did not test around ovulation when hormonal levels may have played a role. Another limitation, and potential cause for discrepancies, could be the lack of consistency in methods used to track the menstrual cycle. Sims and Heather (2018) recently proposed that investigations should include a three-step method to track the menstrual cycle: collection and evaluation of serum hormone concentrations, cycle mapping and urinary ovulation prediction.

Furthermore, when investigating increased muscle recovery, the literature is equivocal. Hackney, Kallman, and Aġgön (2019) investigated muscle damage during recovery of trained individuals during two phases in the menstrual cycle (mid-follicular and mid-luteal). After running on a treadmill for 90 minutes at 70% maximum rate of oxygen consumption (VO_{2max}), creatine kinase (CK) activity and interleukin-6 (IL-6) concentration was greater in the mid-follicular phase than mid-luteal phase at both 24 and 72 hours into recovery. Moreover, IL-6 was significantly greater in the mid-follicular phase compared to the mid-luteal phase, immediately post exercise and at 24 hours and 72 hours into recovery. The results suggest that during the mid-follicular phase, when oestrogen and progesterone are low, muscle recovery takes longer after endurance performance. In contrast, when investigating recovery in strength/resistance training, Markofski & Braun, (2014) found that recovery post exercise was better in the follicular group (when hormones are low). The study suggested that their findings need further investigation. However, it is of interest that they

proposed that oestrogen concentration during the recovery week could be more beneficial compared to oestrogen concentration at the time of muscle damage. In other words, the phase (and hence hormone levels) during the first few day's post-exercise may be more important, and this may not necessarily be the same phase in which the exercise took place (for example, if this occurred near to the transition into the next phase). Another suggestion could be that there was insufficient oestrogen to aid recovery. Refer to section 'rewards and motivation' for more information on how muscle function/recovery may influence an individual's perceptions on how they feel about exercise throughout the menstrual cycle, in regards the motivation/readiness to train.

Thermoregulation

It has been suggested that both oestrogen and progesterone influence thermoregulation through their influence on the hypothalamus (Baker, Waner, et al., 2001; Morrison & Nakamura, 2019). In a typical menstrual cycle (figure 2), a biphasic curve can be seen in basal body temperature (BBT) (temperature at rest) with lower temperature in the follicular phase ($<36.5^{\circ}\text{C}$) and sustained, higher temperatures in the luteal phase ($\sim 36.7^{\circ}\text{C}$); the difference has been shown to be $\sim 0.3 - 0.5^{\circ}\text{C}$ between the two phases) (Baker, Mitchell, & Driver, 2001; Baker, Waner, et al., 2001; Cagnacci, Arangino, Tuveri, Paoletti, & Volpe, 2002; Marshall, 1963; Stephenson & Kolka, 1999; Vidafar et al., 2018). Moreover, ~ 1 day prior to the LH surge, BBT reaches its lowest point before increasing into the early luteal phase (Coyne, Kesick, Doherty, Kolka, & Stephenson, 2000; Stephenson & Kolka, 1999). As progesterone levels drops in the late luteal phase, BBT returns to the lower range $\sim 1 - 2$ days before the onset of menstrual bleeding (de Mouzon, Testart, Lefevre, Pouly, & Frydman, 1984; Su, Yi, Wei, Chang, & Cheng, 2017). The BBT and the biphasic curve can be used to retrospectively detect ovulatory cycles and thus predict phases of the menstrual cycle (Bull et al., 2019; Kawamori, Fukaya, Kitazawa, & Ishiguro, 2019; Tenan et al., 2016; Tenan, Peng, Hackney, & Griffin, 2013).

It is suggested that progesterone is responsible for the hyperthermic effect seen in the luteal phase (Isreal & Schneller, 1950; Stachenfeld, Silva, & Keefe, 2000), as it occurs alongside the progesterone peak. Whereas, oestrogen is responsible for a hypothermic effect, highlighted when oestrogen levels increase and temperature reaches its lowest point (Coyne et al., 2000; Stephenson & Kolka, 1999). The decrease in temperature is thought to be

through the enhancement of vasodilation for heat loss and sweat (Brooks et al., 1997; Charkoudian, Stephens, Pirkle, Kosiba, & Johnson, 1999).

Moreover, Cagnacci et al. (2002) investigated the progesterone/estradiol ratio and found that it was the best predictor variable of body temperature changes throughout the menstrual cycle when compared to investigating progesterone and/or oestradiol separately. Grant et al. (2020) investigated the effects of progesterone and the progesterone/estradiol ratio on core body temperature between the follicular and luteal phases. They found that progesterone and the progesterone/estradiol ratio were significantly associated with core body temperature ($P=0.02$ and 0.01 , respectively). Other researchers have however, disputed progesterone's hyperthermic effect due to the poor correlation between BBT and progesterone concentration (Forman, Chapman, & Steptoe, 1987; Kesner, Wright, Schrader, Chin, & Krieg, 1992; Morris, Underwood, & Easterling, 1976). Additionally, if researchers have not measured hormonal concentrations alongside BBT, it therefore cannot be assumed that the hormone concentrations vary in relation to the BBT values. BBT can also be influenced by lifestyle factors such as stress, alcohol consumption, sleep, diet, nutritional factors, medications and illness (Barron & Fehring, 2005). There are many ways to measure and interpret BBT: specialized charts, online services or smart-phone applications, with some of the interpretations not being clear. BBT is however an easy, non-invasive tool to measure the menstrual cycle but interpretation might be challenging, and it would be best to combine this methodology with another form of testing to validate results (Barron & Fehring, 2005; Bauman, 1981; Bouchard & Genuis, 2011).

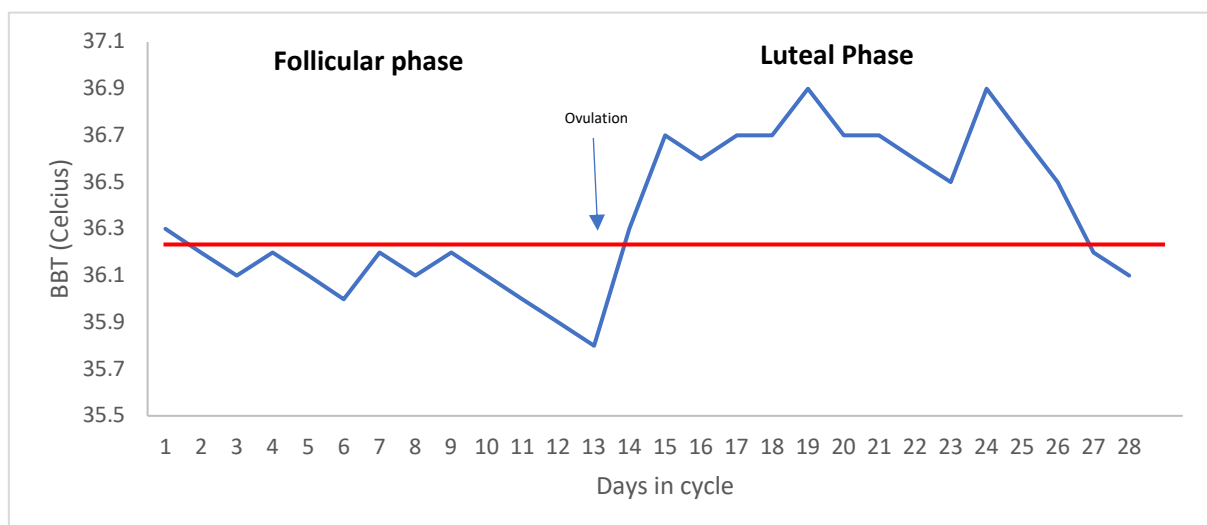


Figure 2. BBT over a typical 28-day menstrual cycle

The menstrual cycle and psychological effects

Overview

Throughout the menstrual cycle, women can experience changes in their physical and psychological symptoms (Romans, Clarkson, Einstein, Petrovic, & Stewart, 2012). Oestrogen and progesterone have receptors localised in the amygdala, hippocampus, cortex and basal forebrain; regions of the brain involved in emotional and cognitive regulation (Wharton, Gleason, Sandra, Carlsson, & Asthana, 2012). The sex hormones have been observed to modulate the synthesis, release and metabolism of several neurotransmitters including serotonin, dopamine, noradrenaline and melatonin. The hormones have also been observed to modulate synaptic functions. Serotonin, a neurotransmitter, has a variety of roles within the body, including regulating the body's sleep wake cycle and an individual's wellbeing and happiness; higher levels of serotonin are associated with increased feelings of wellbeing and happiness (Rybaczuk et al., 2005). Oestrogen and progesterone have been seen to play a role in serotonergic function (Barth, Villringer, & Sacher, 2015). Oestrogen has been observed to upregulate serotonin synthesizing enzyme (tryptophan hydroxylase messenger ribonucleic acid [mRNA]), and downregulate serotonin reuptake transporter mRNA, which suggests that oestrogen can influence serotonin availability and concentration within the central nervous system (Aggarwal, Puri, & Puri, 2012). This can be seen through the use of serotonin reuptake inhibitors for reduction of premenstrual syndrome (PMS) symptoms (Shah et al., 2008). Moreover, high oestrogen levels have been shown to reduce anxious behaviour (Walf & Frye, 2007). On the other hand, progesterone has been observed to increase serotonin turnover, reducing serotonin concentration and availability, which might be related to increased depressive symptoms (Barton et al., 2008), anxiety and irritability (Andréen, Bixo, Nyberg, Sundström-Poromaa, & Bäckström, 2003; MacQueen & Chokka, 2004; Saunders & Hawton, 2006).

Affective and somatic symptoms

Mood changes have been found to occur in both menstrual phases (follicular and luteal) (Boyle & Grant, 1992; Brahmbhatt, Sattigeri, Shah, Kumar, & Parikh, 2013; Van Den Akker & Steptoe, 1985). Psychological changes associated with the menstrual cycle have been investigated, although findings are equivocal

PMS is defined as the ‘reoccurrence of negative behavioural, psychological and physical symptoms’ that occur during the luteal phase of the menstrual cycle (Dickerson, Mazyck, & Hunter, 2003; Farage, Osborn, & MacLean, 2008; Freeman, 2003; Gnanasambanthan & Datta, 2019; Kessel, 2000; Ryu & Kim, 2015). PMS symptoms are sometimes severe enough to interfere with an individual’s daily activities. Brahmhatt et al. (2013) found that 80% of women reported mild symptoms with 20-50% reporting moderate and ~5% reporting severe, similar findings have been found by Moos (1968). PMS symptoms are common within the general population (Brahmhatt et al., 2013) and elite athlete population (Findlay, MacRae, Whyte, Easton, & Forrest, 2020), and tend to worsen 6 days prior (mid-late luteal phase) and peak ~2 days prior to the onset of menses (Kimberly Ann Yonkers, O’Brien, & Eriksson, 2008), with symptoms subsiding into the early follicular phase (Ross, Coleman, & Stojanovska, 2003). Cohen, Sherwin, & Fleming (1987) found that moods were less positive in the luteal phase compared to the follicular phase, with the lowest moods evident on premenstrual days. Symptoms of PMS / menstrual cycle symptoms can include mood and behavioural symptoms (affective symptoms) and somatic symptoms, including but not limited to breast tenderness, bloating, muscle pain, fatigue, and low energy (see table 1). The way in which the symptoms originate is still unclear, however, it is believed that the fluctuations in the sex hormones, oestrogen and progesterone, play a role.

Ross et al. (2003) investigated the patterns of the menstrual cycle in 181 women, either with or without HC use. Each participant completed a modified menstrual distress questionnaire (MDQ) every evening daily for 70 days. The authors standardised each menstrual cycle into a 28-day record, by a mathematical interpolation, and scores were derived by averaging the symptom scores as followed for each phase: follicular days 7 – 11, late luteal days 24 – 28 and menstrual period days 2 – 4. In women without HC use, the authors demonstrated that symptoms were at their lowest during the follicular phase and increased in the premenstrual phase. Somatic symptoms, which include backache, cramps and general aches and pains, were significantly higher during the menstrual phase compared to the premenstrual phase whereas fluid retention was significantly higher in the premenstrual phase compared to the menstrual phase. Similar results were reported by Heitkemper & Jarrett (1992). The study investigated a variety of parameters including mood symptoms, somatic symptoms and perimenstrual symptoms across two menstrual cycles. They separated the phases as followed: menses (days 1 – 5), follicular phase (days 7,8 & 9 post onset of menses) and luteal (days -

10, -9 & -8 prior to onset of next menses). They found that MDQ-T scores were higher for 7 out of 8 subscales (arousal was lower) at menses compared to the follicular and luteal phase. Further analysis showed that menses scores were higher for all but the control and arousal subscales compared to the follicular. Moreover, menses scores were significantly higher than the luteal phase for pain, behaviour change, impaired concentration and autonomic reactions. Lu (2001) assessed individuals using a prospective diary (Woods daily health diary) and using a retrospective questionnaire (MDQ) over a 90 day period (or 3 menstrual cycles). The Woods daily health diary was split into three phases: premenstrual (7 days prior to onset of menses), menstrual (all bleeding days) and postmenstrual (all remaining days of cycle). The MDQ captured three cycle phases: 4 days prior to menses, days of the most recent menses and the remainder of the cycle. Upon analysis of the daily diary, Lu (2001) found that mean values for abdominal pain, cramps and general aches and pains were significantly higher during the menstrual phase compared to the pre and post menstrual phase, whereas symptoms of hostility were significantly higher during the premenstrual and menstrual phase compared to the post menstrual phase. The retrospective MDQ highlighted that over 60% of women reported suffering from physical, psychological and behavioural changes (i.e., fatigue, cramps, mood swings and taking naps) during the menstrual cycle and over 60% reported suffering from painful or tender breast and fatigue during the premenstrual phase. Moreover, it was noted that fatigue was the most frequent and severe symptoms during the premenstrual and menstrual phase with cramps and abdominal pain the second most frequent symptom during the menstrual phase. It is also important to highlight that 3.3% of women reported symptoms that included loneliness, fatigue and avoidance of social activity during the menstrual phase. Li, Lloyd, & Graham (2020) however found that physical fatigue, assessed through a fatigue and energy scale, remained unchanged between the early follicular phase (days 10 – 14 prior to ovulation) and the mid luteal phase (days 5 – 10 after ovulation) whereas mental fatigue significantly increased from the early follicular to mid-luteal phase in non-anxious women. Mental fatigue remained unchanged in women in generalised anxiety disorder, which may suggest that hormonal levels differ in women with and without generalised anxiety disorder, which may indirectly or directly influence fatigue.

Similarly, Gonda et al. (2008) found that during the late luteal phase (2-3 preceding the onset of menses) scores were significantly higher in anxiety and depression when compared to the early and late follicular phase. Similar results are echoed by Abraham, Luscombe, & Soo (2003). Ziomkiewicz et al. (2012) studied 122 regular menstruating females. The females

were asked to record their mood changes throughout one menstrual cycle. The individuals collected morning saliva samples at home, which were analysed for progesterone concentration. The study found that over 80% of the females reported negative mood symptoms during the luteal phase, with 75% reporting an increase in aggressive, irritable, and depressive behaviour during this phase. When looking at the saliva analysis, the study reported that women who reported lower intensities for aggressive or irritable behaviour had significantly higher levels of progesterone throughout the entire luteal phase when compared to women who reported higher intensities of aggressive/irritable behaviour.

Aggressive/irritable behaviour was defined as the ‘sum of intensity of symptoms such as anger and irritability’. However, the study did not find any statistically significant differences in progesterone levels between women who had high and low intensity depressive behaviour. When progesterone levels are high in the luteal phase, Conway et al. (2007) found that individuals had a greater tendency to perceive fearful expressions as more intense (Toffoletto, Lanzenberger, Gingnell, Sundström-Poromaa, & Comasco, 2014). Similar results were found by Andreano and Cahill, (2010) who studied 17 naturally cycling females. The study conducted testing during two phases of the menstrual cycle (early follicular phase and mid-luteal phase). During functional magnetic resonance imaging (fMRI) scans, focusing on the amygdala responsiveness, the individuals were exposed to negative emotional and neutral images. The study found that during the mid-luteal phase, response to negative emotional and neutral images were significantly greater than during the follicular phase. Similar observations were found by Freemas et al. (2020), suggesting and reinforcing that the hormonal balance plays a role in the individuals’ emotions.

A few studies, however, have found that women who suffer from higher anxiety reported more severe symptoms regardless on the menstrual cycle phase (Craner, Sigmon, & Young, 2016; Nillni, Rohan, Mahon, Pineles, & Zvolensky, 2013; Sigmon, Dorhofer, Rohan, & Boulard, 2000). Additionally, in a review of prospective data studies surrounding the mood and the menstrual cycle, Romans, Clarkson, Einstein, Petrovic, and Stewart (2012) suggests that many studies contain misinformation. The aforementioned study suggests that menstruation may have negative connotations and therefore influence individuals’ attitudes, mood, emotions and physical pain. For example, there have been a few studies which have investigated females’ expectations of PMS / menstrual cycle symptoms. Marván and Escobedo (1999) had two groups of females: 1 group watched a negative video of PMS, and the control group watched a video describing the menstrual cycle. The group that watched the

negative video reported increased PMS symptoms compared to the control group. Similar findings have been suggested by Ruble (1977). It is therefore important to take into account individuals, their attitudes, beliefs and baseline measurements, when comparing data (Marván, Vázquez-Toboada, & Chrisler, 2014).

Table 1. Menstrual cycle symptoms

Affective symptoms	Somatic symptoms
Anger	Headache
Anxiety	Tender/swollen/painful breasts
Social withdrawal	Water retention
Confusion	Abdominal bloating
Depression	Joint or muscle pain
Irritability	Low energy / fatigue
Poor concentration	Constipation or diarrhoea

Rewards & motivation

Motivation is defined as “direction and intensity of effort” typically in an effort for a reward (Weinberg & Gould, 2007). Dopamine is a catecholamine neurotransmitter (Fernstrom & Fernstrom, 2007), with dopaminergic neurons located in the substantia nigra and extending to the dorsal striatum, making up the dopaminergic pathways. Dopamine receptors are expressed in the central nervous system (CNS) and also found peripherally in blood vessels, kidneys, heart, retina and adrenals which control catecholamine release. Dopamine plays a role in our behaviour and physical function such as motivation and learning (Hikosaka, Nakamura, Sakai, & Nakahara, 2002; Salamone & Correa, 2012; Westbrook & Braver, 2016), sleep (Monti & Monti, 2007; Wisor et al., 2001), mood movement/locomotor activity (Harrington, Augood, Kingsbury, Foster, & Emson, 1996), endocrine regulation and food intake. As mentioned previously, steroid sex hormones, oestrogen and progesterone, have been observed to interact with dopamine. Oestrogen has been seen to increase an individual’s reactions to reward stimuli by increasing synthesis and release of dopamine (Sakaki & Mather, 2012). However, when progesterone levels are high, oestrogen receptors are downregulated. Therefore, it is plausible to assume that during the follicular phase compared to the luteal phase, an individual’s reaction and effort to reward is a lot stronger (Justice & De Wit, 2000; White, Justice, & De Wit, 2002; Yoest, Cummings, & Becker, 2015).

Moreover, testosterone, a sex hormone, is involved in voluntary motivation. Testosterone levels increase prior to ovulation and prior to onset of menses. Cook and Beaven (2013) demonstrated that increased levels of testosterone were linked with increased motivation to train. Similar results were concluded by Cook, Kilduff, and Crewther (2018) who showed that females had a higher motivation to train on day 14 (near ovulation) compared to the mid-follicular and mid-luteal phase. Furthermore, a review completed by Yesildere Saglam and Orsal (2020) demonstrated the effect of exercise on PMS with many studies showing positive influences on improving physical and psychological symptoms. However, PMS / menstrual cycle symptoms like fatigue, tiredness and decreased mood can affect motivation to complete exercise in the days leading up to the follicular phase (Lustyk & Gerrish, 2010; Takeda, Imoto, Nagasawa, Muroya, & Shiina, 2015).

The hormonal fluctuations seen throughout the menstrual cycle could contribute to the individuals self-reported perceptions of how they feel about training or exercising, in relation to their readiness/motivation to train. Armour, Parry, Steel, and Smith, (2020) found that athletes experienced perceived reductions in performance, with increased fatigue and reduced strength, speed and agility during or just prior to the onset of menses. Moreover, Brown, Knight, and Forrest (2020) found that athletes often felt ‘sluggish during training’ due to feeling lethargic, pre and/or during their menses. Interestingly, the authors noted that a large proportion of participants often lacked the motivation to train as they would rather do more passive activities, which required less energy, or nothing at all. Lack of motivation to train was often down to having no energy or physical symptoms such as pain/bloating impacting performance. The individuals preference for low intensity and/or low energy requiring exercise or none at all may be due to the additional perceived effort that is needed to carry out exercise pre/during menses. Hooper, Bryan, and Eaton (2011) discovered that during the early follicular / menses phase, females had significantly greater increases in ratings of perceived exertion when completing exercise.

Hormonal contraceptives

Overview

HC's suppress the normal menstrual cycle hormones, via negative feedback, by inhibiting hypothalamic release of gonadotrophin-releasing hormones (Elliott-Sale et al., 2013), which results in the downregulation of endogenous oestrogen and progesterone (Elliott, Cable, & Reilly, 2005), decreased production of testosterone (Zimmerman, Eijkemans, Coelingh Bennink, Blankenstein, & Fauser, 2014) and inhibition of ovulation. There is a variety of HC, including the combined oral OC, which contains synthetic exogenous oestrogen (ethinyl estradiol (EE) at 15 – 35 µg) and progestin (Hampson, 2020) and a progestin-only contraceptive. Combined OCs are the most commonly used within the athletic population (Martin et al., 2018; Rechichi, Dawson, & Goodman, 2009). There are three types of combined OCs: monophasic, biphasic and triphasic. The monophasic OC have a constant dose of exogenous oestrogen and progestin over 21 days (OC consumption phase), with a 7-day period involving no pill or a placebo pill (OC withdrawal phase) (see figure 3). Monophasic exogenous hormone half-lives should be noted, as it takes 7 days of pill consumption to completely downregulate the pituitary glands and 5 days of OC withdrawal to stabilise progestin levels (Elliott et al., 2005). Creinin, Lippman, Eder, Godwin, and Olson (2002) suggested that the withdrawal phase should be termed a transient hormonal profile phase due to this. The biphasic and triphasic OC hormone doses may vary throughout the cycle. The 'controlled' menstrual cycle lasts 28 days (Redman & Weatherby, 2004), with a withdrawal bleed (artificial period) occurring during the pill withdrawal phase. There are several brands of combined OCs, each containing different doses of the two synthetic hormones and different potencies of progestin (Burrows & Peters, 2007; Christin-Maitre, 2013; Elliott-Sale et al., 2013).

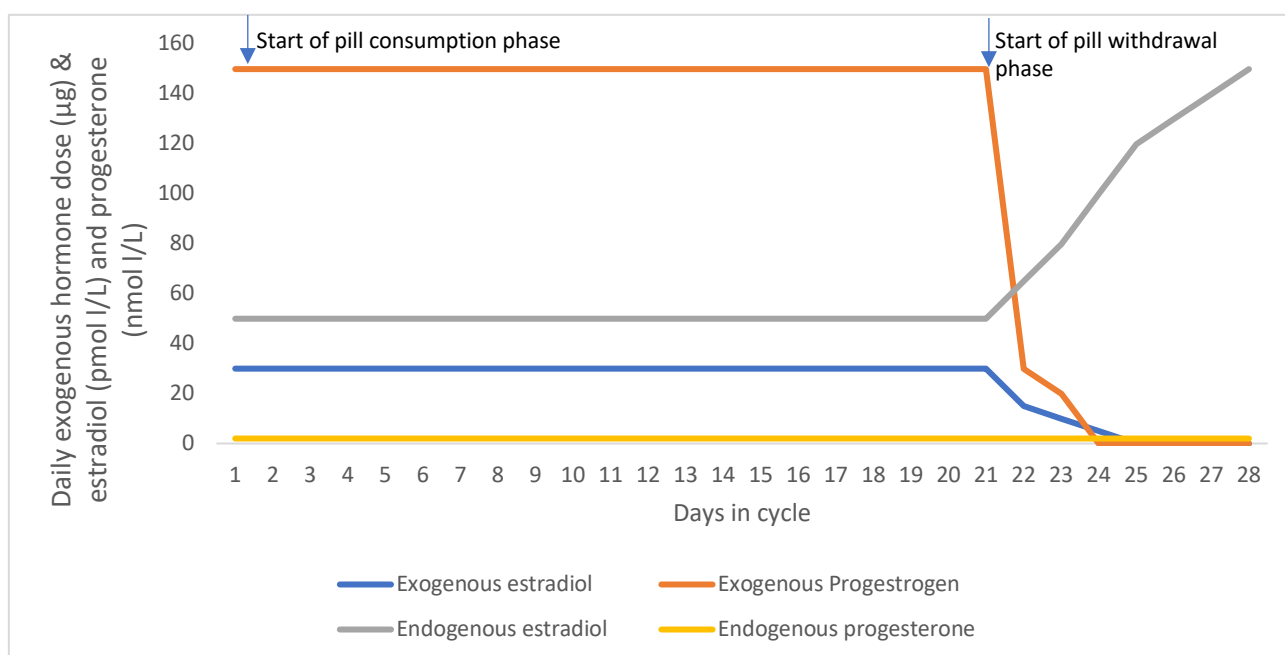


Figure 3. Exogenous and endogenous hormones throughout the menstrual cycle for females using monophasic oral contraceptives.

Key hormones: oestrogen & progestin

As mentioned previously, OCs contain two synthetic exogenous hormones: the oestrogen component is estradiol and progesterone component is progestin. Since OCs were first released, the OC generations have changed formulations. The OC generation relies on the type of progestin used; the newer generations (second and third) contain different doses of both hormones and the progestin has lower androgenic properties to reduce / decrease potential side effects such as acne and oily skin. As OCs downregulate endogenous hormones, inhibit ovulation occurring and help stabilise the hormonal levels, it is hypothesized that the physical, psychological, and emotional symptoms that are associated with hormonal fluctuations do not occur or are reduced.

Thermoregulation

As mentioned in the thermoregulation section of non-OC users, the steroid hormones, oestrogen and progesterone, have been seen to play a role in thermoregulation, with increases in BBT during luteal phase of the menstrual cycle. Several studies have investigated whether the synthetic steroids in OC influence thermoregulation in a similar manner. Baker, Mitchell, and Driver (2001) investigated 11 monophasic OC users and 12 non-OC users. Endogenous hormone concentrations were the same in the pill consumption and pill withdrawal phase

and, as expected, were significantly lower than naturally cycling women in both phases. The study found that during a 24 hour recording period, OC users had a similar mean temperature across both phases (pill and withdrawal, 37.4°C), with temperatures similar to those of non-oc users in the luteal phase (37.4°C). Similarly, Baker et al. (2001) discovered that during a 24 hour recording period, monophasic OC users during the pill consumption phase had similar endogenous hormone concentrations to those of non-oc users in the follicular phase (both hormones low). Moreover, temperatures during the pill consumption phase were similar to those of non-oc users during the luteal phase (37.5°C and 37.4°C, respectively). It is therefore reasonable to assume that OC users do not follow the same temperature pattern compared to non-oc users. There are a few mechanisms of action which may be responsible for this. Rogers and Baker (1996) suggested that the increase in temperature throughout both phases (pill consumption and withdrawal) may be due to the synthetic hormones increasing the body's core temperature. In contrast, Lei et al. (2019) compared days 3-5 and days 18-20 following start of monophasic OC consumption. Their findings demonstrated that rectal temperature was significantly higher during days 18-20 compared to days 3-5 (0.15°C difference), alongside this, both endogenous and exogenous hormone concentrations of oestrogen and progesterone did not differ between the two time points, suggesting that neither endogenous nor exogenous played a role in the temperature increase. Although, it should be highlighted that the temperatures were recorded at rest and not measured as BBT measurements (i.e., in the morning upon waking).

It should be noted that it can take up to 5 days of OC withdrawal to stabilise progestin levels and therefore the standardisation of 'at least 3 days' after pill withdrawal could lead to inaccurate findings in Baker, Mitchell, et al. (2001) investigation. It is of interest to also point out that studies like the one mentioned and Baker, Waner, et al. (2001) did not standardise the day(s) within the consumption phase when recordings were carried out, which again, may influence results collected.

Stachenfeld et al. (2000) investigated the effects of combined oestrogen and progestin and of progestin only oral contraceptives on nine healthy women. Individuals' baseline measurements were assessed in the early follicular phase and midluteal phase, without OC consumption. Each treatment group lasted four weeks, with a four week washout period before subjects crossed over to the other treatment. Body core temperature was measured from an oesophageal thermocouple. The study found that when progestin only was

administered, there was an increase in core temperature and threshold for sweating, however, when oestrogen was administered with progestin, the effects were reversed. It should, however, be highlighted that oestrogen's hypothermic effect may only be temporary, as progesterone's hyperthermic effect is dominant (Isreal & Schneller, 1950). It should be noted that four weeks of OC consumption may not be long enough to allow the body to adjust to the exogenous hormones. The mechanisms of action are still unclear; however, it is likely that the steroids influence the hypothalamus in a similar manner to the endogenous sex hormones.

Oral contraceptive use and psychological effects

Overview

It is thought that the downregulation of endogenous hormones may stabilise and possibly reduce the effects the menstrual cycle has on the body. There is however no consensus within the literature, with some studies suggesting that OCs may have a negative effect (Robinson, Dowell, Pedulla, & McCauley, 2004), no effect (Bruni et al., 2000; Marriott & Faragher, 1986) or a positive effect (Boyle & Grant, 1992) on affective and/or somatic symptoms (Graham, Bancroft, Doll, Greco, & Tanner, 2007; McKetta & Keyes, 2019; Robakis, Williams, Nutkiewicz, & Rasgon, 2019). The lack of consensus may be due to the variety of OCs, the dose of oestrogen and progestin and progestins potency (as discussed previously) as well as methodological differences used within the literature (Oinonen & Mazmanian, 2001; Schaffir, Worly, & Gur, 2016).

Affective and somatic symptoms

HC's have been thought to help reduce levels of depressive symptoms (Keyes et al., 2013). Joffe et al. (2007) found that females taking monophasic OCs (30 µg EE and 3mg drospirenone) alongside their antidepressant medication significantly helped treat premenstrual depression, which is seen in the luteal phase of the cycle. Toffol, Heikinheimo, Koponen, Luoto, and Partonen (2011) studied OCs, their results highlighted lower but not significant scores of the Beck Depression Inventory for OC users compared to non-OC users, suggesting that OCs did not have detrimental effects on mood. Similar findings were suggested by Toffol, Heikinheimo, Koponen, Luoto, and Partonen (2012) and McKetta and Keyes (2019). Boyle and Grant (1992) also demonstrated that when asked about their beliefs about the

effect of the OC on menstrual cycle mood and symptoms, 65% of OC users felt as though their psychological moods were reduced by the OC use, although it should be noted that the OC type/brand was not disclosed. Similarly, the results could be due to a placebo effect, i.e., participants believe that using OC's will reduce their symptoms compared to testing with and without OC. Interestingly, the study completed further analysis on the menstrual attitude questionnaire and found that the non-oc users did report menstruation as significantly more debilitating than the oc users, although none of the other items measured showed any significant difference. When analysing the MDQ, oc users did report significantly fewer symptoms and negative moods than non-oc users. The findings may be due to the OC stabilising the hormonal levels through the more 'controlled' and 'steady' exogenous hormones throughout the cycle and therefore 'protecting' the individual from their endogenous hormones and what effect they may have on mood. This can be seen through Abraham, Luscombe, and Soo (2003) study whereby non-OC individuals had a sharp premenstrual increase in sadness/depression frequency compared to OC-users (Natale & Albertazzi, 2006). Alternatively, it has been suggested that OC users experience negative psychological side effects due to the exogenous hormones. Zethraeus et al. (2017) investigated individuals taking a monophasic OC for three months (30 µg EE & 150 µg levonorgestrel and) and found that individuals who used the OC compared to non-oc users had a significant reduction (6%) in general well-being from baseline to 3 months post OC consumption. However, it should be noted that there was no significant difference in negative mood/depressive symptoms in OC users compared to non-OC users in the study. A possible mechanism of action could be that exogenous progestin having a similar effect to endogenous progesterone; exogenous progestin has been observed to reduce serotonin concentration and availability, which therefore negatively affect an individual's mood (Holst, Bäckström, Hammarbäck, & von Schoultz, 1989; Klaiber, Broverman, Vogel, Peterson, & Snyder, 1996). This theory is reinforced through the increased risk of depression in progestin only pills (Skovlund, Mørch, Kessing, & Lidegaard, 2016; K. Smith et al., 2018). Furthermore, the differences in findings could be due to the individuals investigated. Garbers, Correa, Tobier, Blust, and Chiasson (2010) highlighted that individuals who have higher depressive symptoms or are screened positive for depression are less likely to use exogenous contraception methods, including OCs and therefore if individual's with lower levels of depression are investigated in the studies, it is likely that their depression scores will be lower (Joffe, Cohen, & Harlow, 2003; Keyes et al., 2013).

Mood lability (changes in mood) has also been investigated. Ott, Shew, Ofner, Tu, and Fortenberry (2008) demonstrated that OCs, when compared to depot medroxyprogesterone acetate (a HC), helped reduce variation in mood. Similar results were concluded by Taggart, Eaton, Keyes, Hammett, and Ulloa (2018) who compared OC users and Non-OC users. Their study found that OC users reported a 41 % presence of mood lability compared to non-OC users reporting 63 %. Although, it should be noted that the study did not report what type/brand of OCs were studied. Graham, Bancroft, Doll, Greco, and Tanner (2007) studied individuals pre- and post-3 months of taking a triphasic OC. The results concluded that there was no significant change in mood due to the triphasic OC. Upon closer inspection of the data however, large groups can be seen in each of the following categories: no change (19), positive (17) and negative change (24). The results highlight the variety of individual responses, which may suggest it is up to the individuals as to how they respond to OC use (Oinonen & Mazmanian, 2002; Robakis et al., 2019). Moreover, the equivocal findings could also be due to the use of different generations of OCs within the studies. Shahnazi et al. (2014) studied second and third generation OC's and their effect on mood. The study concluded that third generation OCS are better for mood stability than second generation due to the lower potency of progestin. It is therefore challenging to draw conclusions between studies when a variety of OC generations with various progestin levels have been used and/or the OC generations/types have not been disclosed.

Nyberg (2013) investigated women who suffered with or without PMS symptoms. All subjects were placed on monophasic OC (35 µg EE / 250 µg norgestimate). The study found that females who suffered with severe PMS showed improved mood symptoms although females who showed no or mild PMS symptoms did not show any significant improvement or decline in symptoms. Sanders, Graham, Bass, and Bancroft (2001) studied individuals who had no previous use of OC. The individuals were randomly assigned either a monophasic OC (35 µg EE and 250 µg norgestimate) or triphasic OC (35 EE and 180, 215 µg and 250 µg norgestimate). The study assessed individuals prior to starting their OC use and reassessed at 3, 6 and 12 months. During the initial assessment, prior to OC use, 57 % of individuals believed they suffered from PMS. When individuals were asked what the OCs effect was on PMS after OC use, more females perceived that it helped alongside this, those that perceived the pill made PMS better also indicated that OC use reduced menstrual pain. Similarly, Boyle and Grant (1992) who demonstrated that 79% of OC users believed that physical symptoms reduced with OC use, although this is based on perceptions rather than testing which could

increase possibilities for expectancy effects. Abraham, Luscombe, and Soo (2003) found that non-OC users reported more frequent tiredness and fatigue compared to OC-users. It should be noted that differences in OC formulations could affect the conclusions drawn between the literature, with monophasic and triphasic formulations potentially having different effects on somatic and/ or affective symptoms. Ross, Coleman, and Stojanovska (2003) investigated this theory and found that somatic symptoms and fluid retention were reported at a higher rate for monophasic OC users. Although, Bruni et al. (2000) found that the adverse effects of monophasic and triphasic OC users were similar.

Moreover, during the withdrawal week, several studies have noted that although OC users reported a lower severity of symptoms compared to non-oc users, they still experienced changes throughout the 28-day cycle, particularly during the premenstrual and menses phase (Adan, Prat, Fabbri, & Sánchez-Turet, 2008; Coffee, Sulak, & Kuehl, 2007; Hamstra, de Kloet, de Rover, & Van der Does, 2017; Sulak, Kuehl, Ortiz, & Shull, 2002; Yonkers, Cameron, Gueorguieva, Altemus, & Kornstein, 2017), it is therefore of interest to look into the cyclic changes that may occur during the 28 day period, looking at comparing both the consumption and withdrawal period and within the withdrawal period (the start and the end).

Rewards & motivation

Oral contraceptives have the ability to reduce testosterone levels within the body, by increasing levels of sex-hormone-binding globulin (SHBG), which decreases free testosterone levels, alongside altering other physiological processes (Greco, Graham, Bancroft, Tanner, & Doll, 2007; Vibarel-Rebot, Rieth, Lasne, Jaffré, & Collomp, 2015; Zimmerman et al., 2014). As mentioned within the non-oc section, testosterone is involved in motivation and therefore it would be reasonable to assume that low levels of testosterone are associated with lower levels of motivation to train and higher levels of perceived effort to train (Cook & Beaven, 2013; Crewther, Hamilton, Casto, Kilduff, & Cook, 2015). Moreover, as mentioned within the non-oc literature review if females experience premenstrual and menstrual symptoms this would also have an impact on their motivation to train.

Methods for validating menstrual cycle phase

Tracking the menstrual cycle is important, as it helps to determine which phase the female is in. However, it should be noted that even in eumenorrheic females regular hormone concentration fluctuations cannot be assumed and therefore testing is vital to help validate the hormones (Scheid & De Souza, 2010).

Self-report/calendar-based counting

Calendar-based counting is an indirect self-reported onset of menses, which helps to determine the menstrual phases by counting the onset of menses as day 1 of the cycle and then counting the days from that point. Many studies have used self-report/calendar-based counting methodology to help track the menstrual cycle (De Jonge et al., 2019); with most studies using it as supplementary information alongside other scientific methodology to confirm cycle phase, as self-reports can be unreliable and lack in validity (Johnson, Miro, Barrett, & Ellis, 2009; Small, Manatunga, & Marcus, 2007; Wideman, Montgomery, Levine, Beynon, & Shultz, 2013). Moreover, self-reports are retrospective in nature, as the days need to be counted backwards to predict phases, which is not useful for a prospective research study where testing is required within certain phases. It is also challenging to estimate when ovulation occurs due to the retrospective nature. Additionally, many studies lack a clear, coherent definition to state the beginning of menses, which is required to eliminate subject error in dating the onset of menses (Slauterbeck et al., 2002; Taffe & Dennerstein, 2000).

Basal body temperature

Since there are predictable changes in BBT across menstrual cycle phases (as discussed in thermoregulation section), it is possible to use regular temperature monitoring (e.g., daily logging of BBT) to predict phases.

Urinary LH measurement

As previously mentioned, ovulation is triggered by a surge in LH (Yoshimura & Wallach, 1987). Detection and tracking of the female ovulation day would help researchers determine when the female has entered the luteal phase (Eichner & Timpe, 2004). The LH surge can be detected through urine, which is a non-invasive and relatively inexpensive procedure

(Gudgeon, Leader, & Howard, 1990; Katler, Tricarico, & Bishop, Lauren, 2018). With this form of methodology, the individual urinates on the test stick or places the stick into collected urine, dependent on the form of ovulation kit. The stick will then turn a certain colour or display a positive sign if LH surge is detected. To detect LH surge, urinary LH tests are used a few days prior to predicted ovulation and continued until a positive result occurs. Once LH surge is detected, ovulation usually occurs within 24 – 36 hours (Miller & Soules, 1996; “What is Ovulation? The What, When and How – Clearblue,” n.d.). A few studies have used this method to help detect ovulation when investigating the effects of the menstrual cycle on sport (Forsyth & Reilly, 2008; Hertel, Williams, Olmsted-Kramer, Leidy, & Putukian, 2006; Romero-Moraleda et al., 2019; Tsampoukos, Peckham, James, & Nevill, 2010). Park, Goldsmith, Skurnick, Wojtczuk, & Weiss (2007) however, advises caution when using urinary LH to detect ovulation. Their study demonstrated that LH surges across 43 women varied in amplitude, duration and configuration. Moreover, they discovered that not all LH surges result in ovulation occurring even if the female has a regular menstrual cycle. The study concluded that, LH surge is defined by ≥ 2.5 -fold increase from baseline and can last a duration of 5 – 11 days. Furthermore, at home testing can result in false-positive results (Ghazeeri, Vongprachanh, & Kutteh, 2000; McGovern et al., 2004) falsely indicating ovulation occurred when it did not. Therefore, it is suggested that other methods are used to help verify menstrual phase.

It is suggested that the methodology used to measure the menstrual cycle changes in a practical setting should be non-invasive, inexpensive, easily available and easy to use (Su et al., 2017). Whereas when in a research setting, the main priority is that the methodology is accurate and valid. It is thought that a combination of different methods might be able to lead to a better understanding of the changes that occur during the cycle (Allen et al., 2016).

Research aims.

The project has 3 aims:

Aim one: to determine if menstrual cycle phase or oral contraceptives use influences body temperature

Aim two: to determine if menstrual cycle phase or oral contraceptives use influences mood and/or somatic symptoms

Aim three: to determine the utility of self-monitoring methods, such as self-report/calendar based, BBT, urine LH measurements.

III. Methodology

Participants

16 participants took part in the study (9 non-OC and 7 OC users). However, only 10 (4 non-OC and 6 OC users) participants met the inclusion criteria for the data analysis. See table 2 for participant characteristics and table 3 for reasons for participant exclusion. The volunteers were recruited through the university of Kent school email system, word of mouth and advertisements. All participants were healthy, free from injury and with no known neurological or cardiovascular diseases. All participants recruited were either experiencing normal menstrual cycles (free from OC use for at least 3 months) or using a monophasic OC for at least 3 months. Written consent was obtained, in line with Declaration of Helsinki, and the study was approved by the research Ethics and Advisory Group of the School of Sport and Exercise Sciences, at the University of Kent (Prop 37_2019_20). Participants logged and tracked their menstrual cycles over 2 – 3 months (or 2 – 3 complete menstrual cycles).

Table 2. Participant characteristics

Variable	Range, Mean \pm SD
Non – OC group. N = 4	
Age (years)	23 – 27, 24.8 \pm 1.7
Height (cm)	158 – 183, 166.8 \pm 11.2
Body mass (kg)	56.2 – 100, 75.8 \pm 18.1
Cycle length (days)	27 – 28, 27.58 \pm 0.42
Follicular phase (days)	12.5 – 17.5 (13 – 18 days), 14.82 \pm 1.10
Luteal phase (days)	10.5 – 14.5 (11 – 15 days), 12.75 \pm 1.73
Menses length (days)	3.5 – 4.7 (4 – 5 days), 4.1 \pm 0.5
First peak (day)	11.5 – 16.5 (12 – 17 days), 13.8 \pm 2.1
EDO (day)	12.5 – 17.5 (13 – 18 days), 14.8 \pm 2.1
LH spike length (days)	1.3 – 4 (1 – 4 days). 2.3 \pm 1.2
Physical activity (minutes/day ⁻¹)	Light intensity – 75 – 599, 264.7 \pm 290.4
	Moderate intensity – 45 – 180, 115.0 \pm 67.6
	Vigorous intensity – 35 – 150, 61.7 \pm 78.5
OC group. N = 6	
Age (years)	20 – 48, 30.2 \pm 9.7
Height (cm)	170 – 182, 171.7 \pm 5.8
Body mass (kg)	60 – 94, 73.8 \pm 12.02
Menses length (days)	3 – 5.3 (3 – 5 days), 4.0 \pm 0.8
Type of OC	Monophasic
Brand of OC	20 – 35 μ g EE / 75 μ g – 3mg of progesterone (gestodene, levonorgestrel, drospirenone, desorgestrel and norgestimate)
Physical activity (minutes/week ⁻¹)	Light intensity – 30 – 240, 103.3 \pm 77.6
	Moderate intensity – 105 – 300, 208.0 \pm 88.2
	Vigorous intensity – 15 – 300, 138.8 \pm 118.3

Table 3. Participant exclusion

Number of participants	Reason for exclusion
2	Anovulatory cycles
4	Data missing

Data collection

Menstrual cycle was assessed through a combination of measures, including basal body temperature, urinary luteinising hormone detection, self-report logs and menstrual history. Participant physical activity was assessed through physical activity logs.

All participants recorded their BBT using an oral digital thermometer (Clinical thermometer, One Step, Home Health UK) upon waking each morning through the entire duration of enrolment. Eumenorrheic participants were instructed to test for a urinary LH ‘spike’ (in order to identify the point of ovulation) at home from day 9 post onset of menses (self-reported) daily for 10 consecutive days. The LH (ovulation) kits (Urine test strip, 20mIU/mL 2.5mm, One Step, Home Health UK) were used to detect a surge in urinary luteinizing hormone that occurs prior to ovulation. Each participant was asked to collect their sample at the same time each day (10:30 am, to minimise potential confounding effects of diurnal variations or at a regular time slot between the hours of 10am – 8pm- i.e., regardless of the time, to ensure that it was the same time each day), and ensured they reduced their fluid intake 2 hours prior to sample collection to reduce potential confounding effects of fluid-induced diuresis leading to overly dilute urine samples. See appendix F.

All participants self-reported their menstrual cycle. Clear guidelines were set to define the menstrual cycle, onset of menstruation and the end of menstruation. The menstrual cycle is defined as the number of days between the first day of menstrual bleeding of one cycle to the day before the onset of menses of the next cycle (NHS, 2016). Onset of menses is defined as day 1 of menstrual bleeding whereby feminine protection (i.e., sanitary towels etc.) is required. This statement was used to try and avoid participant error in dating the onset of menses and help differentiate from spotting (Lovering & Romani, 2005; Slauterbeck et al., 2002). The end of menses is defined as the last day in which feminine protection is needed. Moreover, a time point of 12:00 midday was used to classify which day the cycle starts/end

(e.g., if menses commences before midday, that day is recorded as day 1, and if it commences after midday, the following day is recorded as day 1). See table 4.

Table 4. Defining onset and end of menstruation.

Onset of menses	
If bleeding starts before 12:00 midday	It is classified as day 1 of your period
If bleeding starts after 12:00 midday	The following day is classified as day 1 of your period
End of menses	
If bleeding ends before 12:00 midday	It is classified as end of your period
If bleeding ends after 12:00 midday	The following day is classified as end of your period

Questionnaires

Participants completed a variety of questionnaires (menstrual history, menstrual distress questionnaire, Brunel Mood Scale [BRUMS] and readiness to invest effort) during each menstrual cycle, the days in which questionnaires were completed differed between the two groups (Non-OC and OC-users). See table 5 for overview of study design.

Menstrual history and Menstrual Distress Questionnaire (see appendix B & E)

The menstrual history questionnaire was used gain more information about the individual's menstrual cycle, including the length of the menstrual cycle, the length menses, frequency of cycles oral contraceptive use (and brand) and symptoms experienced during the menstrual cycle. The information was then used to assess if the individual met the inclusion criteria.

The MDQ prospective questionnaire is a multivariate self-report inventory designed to capture the symptoms of psychological changes throughout the menstrual cycle (Moos, 1968). The MDQ comprises of 47-items split into 8 sub scales: pain, water retention, autonomic reactions, impaired concentration, behaviour changes, negative affect, arousal and control. Boyle (1992) has provided evidence of validity of the MDQ. Form T of the MDQ was used to gain a prospective understanding of how the individual is feeling at the present. Each symptom is rated on a 6-point Likert scale according to severity of experience (from 1 being no reaction at all to 6 being acute or partially disabling). The prospective questionnaire was included in the individuals log booklet.

Mood (Brunel Mood Scale) and Readiness to Invest Effort (see appendix C and D)

The 24-items BRUMS questionnaire was used to measure 6 mood states (tension, depression, anger, vigour, fatigue and confusion) through a self-report inventory (Duncan & Oxford, 2011; Terry & Lane, 2003). The current study also measured each participant's readiness to invest physical and mental effort (Midgley, McNaughton, Polman, & Marchant, 2007) in regards to physical activity/training through visual analogue scales ranging from 0-10, with high scores reflecting greater readiness to invest effort (Nyenhuis, Stern, Yamamoto, Luchetta, & Arruda, 1997; Tenenbaum et al., 2005). If participants were not completing physical activity on the day of recording, they were instructed to rate it on a scale as if they were going to do physical activity. Both questionnaires were included in the individuals log booklet.

Table 5. Overview of study design

Eumenorrheic females	OC users
Menstrual history questionnaire	
BBT (every morning)	
Self-report logs – see appendix G and H	
Physical activity logs	
LH test	
From day 9 post onset of menses (self-reported) daily for 10 consecutive days or shorter, if spike detected and then subsided before day 10.	
MDQ prospective	
Every day	Every day of pill withdrawal phase
BRUMS & Readiness to invest effort	
Days 2 – 4 post onset of menses. To represent early follicular /menses phase	Days 8 – 10 of pill consumption phase
Day 9+ until ovulation confirmed. To help estimate day of ovulation	Days 5 – 7 of pill withdrawal phase
+7 days after ovulation confirmed. To represent mid luteal phase	
2 – 3 days prior to onset of predicted next menses. To represent late luteal phase / premenstrual phase	

Statistical analysis

Statistical tests were performed using IBM SPSS Statistic 27 software package (IBM, SPSS software, Chicago, IL) and descriptive data is presented as mean \pm SD. Regarding the Non-OC group, data including, BBT and positive LH, MDQ, Brums and readiness to invest physical and mental effort was analysed using 1-way repeated measures analysis of variance (ANOVA) in an attempt to highlight any differences across the set time points / phases. When significant main effects were identified, post hoc analysis was used. Paired t-tests were used to assess difference in BBT across two phases of the menstrual cycle (follicular phase vs. luteal phase and mid follicular phase vs. mid luteal phase). A significance was accepted when $p \leq 0.05$. Cohen's D was used to measure effect size between means of premenstrual and early follicular phases. The formula used was Cohen's D = mean of group one – mean of

group two divided by pooled standard deviations for two groups ($d = M_1 - M_2 / s_{\text{pooled}}$). Threshold values used were: <0.2 = small effect; 0.5 = medium effect; ≥ 0.8 = large effect.

Regarding the OC group, paired t-tests were used to assess differences across two phases, consumption phase vs. withdrawal phase, in BBT, Brums and readiness to invest physical and mental effort, and between the start of withdrawal phase vs. end of withdrawal phase, in MDQ. A significance was accepted when $p \leq 0.05$.

The data was checked for normality (Shapiro-Wilk test). If this was violated, log-transformations were performed. If log transformations did not normalise data, the Friedman test was performed and the non-parametric Wilcoxon matched-pairs was performed. The following parameters were normalised by log transformation: OC users: behavioural change and anger. The following parameters could not be normalised so were analysed with non-parametric tests: non-OC: autonomic reactions, water retention, anger, depression, fatigue and tension; OC users: pain, autonomic reactions, control, confusion, depression and vigour.

Standardisation of procedures

Follicular phase length was defined as the first day of recorded menstruation to the estimated day of ovulation (EDO) (Bull et al., 2019). The luteal phase length was defined as the day after the EDO to the day before the next day of recorded menstruation. EDO was defined as day after positive ovulation test logged.

IV. Results

Eumenorrheic results

BBT & positive LH test

The 1-way ANOVA revealed no significant effect between BBT and positive LH test ($P = 0.171$). A few participants had multiple positive tests recorded in one cycle. Only the first positive test logged within the cycle was used to EDO, as the duration of the LH surge detected by the LH tests can vary across women.

Majority of participants demonstrated a slight decrease in temperature prior to 1st LH spike (on day -1). Day +4 following EDO, participants temperatures were \geq day -2 in preovulatory phase.

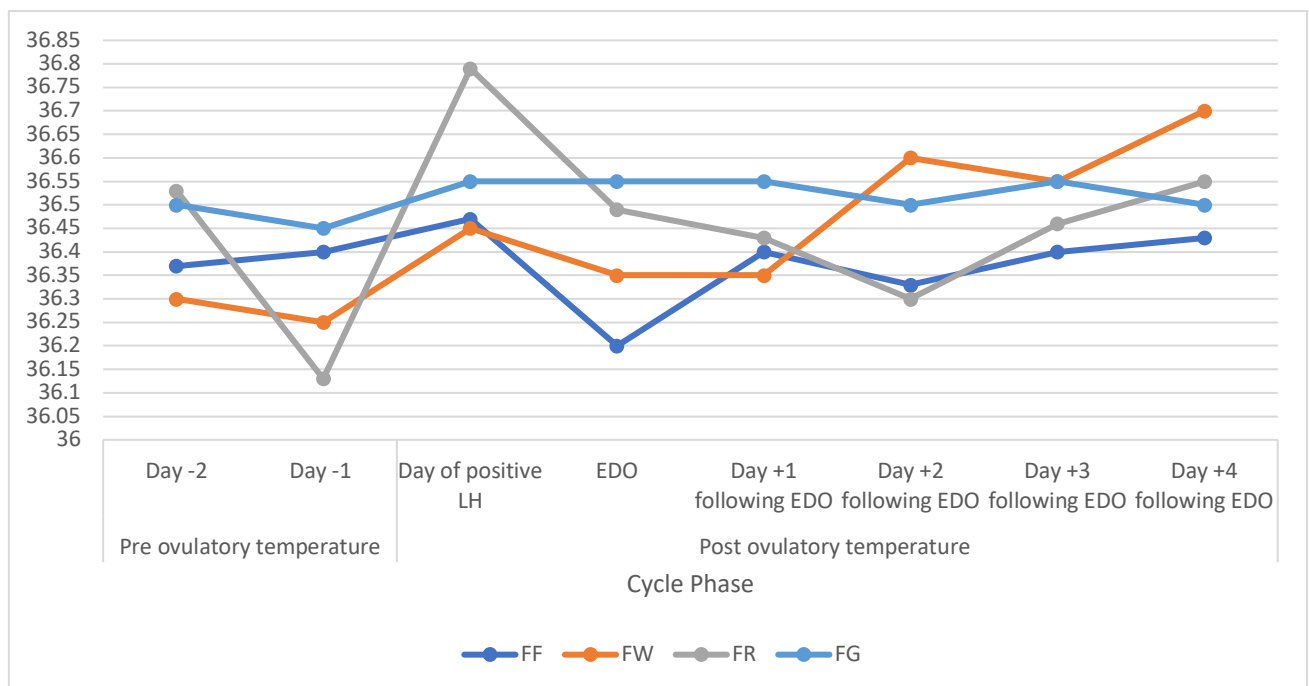


Figure 4 Temperature & positive LH test. Participant ID: FF, FW, FR and FG.

BBT & follicular Vs. luteal phase

A paired samples t-test revealed no significant difference between BBT in the follicular or luteal phase ($P = 0.073$), with the average temperature $36.38 \pm 0.09^\circ\text{C}$ and $36.57 \pm 0.08^\circ\text{C}$ in the follicular and luteal phase, respectively, a difference of 0.19°C .

BBT & Mid follicular Vs. Mid luteal phase

A paired samples t-test revealed no significant difference between BBT in the mid follicular or mid luteal phase ($P = 0.112$), with the average temperature 36.32°C and 36.63°C in the mid follicular phase and mid luteal phase, respectively, a difference of 0.31°C . Mid follicular phase was defined as day -7 to -3 before positive test and mid luteal phase was defined as day +6 to +10 days following positive test, with ovulation day as 0.

Individuals who demonstrated a difference between follicular & luteal phase demonstrated a greater difference comparing the mid follicular phase with the mid luteal phase.

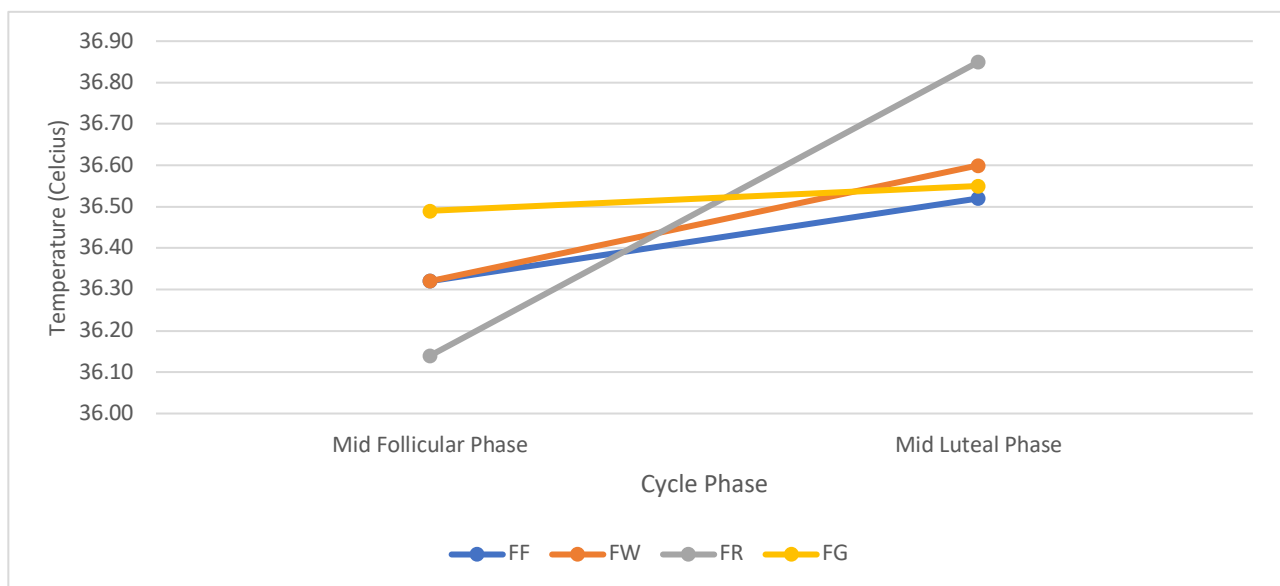


Figure 5 Temperature across the mid follicular and mid luteal phase. Participant ID: FF, FW, FR and FG.

MDQ & premenstrual, early follicular, late follicular and luteal phase.

The MDQ comprises of 47-items, however for the purpose of this study, “change of eating habits” was removed in accordance with the findings of Moos (1968).

Table 6 MDQ across premenstrual early follicular, late follicular and luteal phase

	Menstrual phase			
Subscale	Premenstrual phase	Early follicular phase	Late follicular phase	Luteal phase
Pain				
Mean \pm SD	9.5 \pm 3.2	12.7 \pm 4.4	9.5 \pm 2.8	9.8 \pm 3.2
Range	7 – 14	9 – 18	7 – 13	7 – 14
Concentration				
Mean \pm SD	12.3 \pm 3.1	12.8 \pm 3.9	12.5 \pm 3.3	12.5 \pm 3.4
Range	8 – 15	8 – 17	8 – 16	8 – 16
Behavioural change				
Mean \pm SD	7.8 \pm 2.1	9.0 \pm 2.6	8.6 \pm 2.7	8.0 \pm 2.2
Range	5 – 9	6 – 12	5 – 10	5 – 10
Autonomic reactions				
Mean \pm SD	4.4 \pm 0.8	5.1 \pm 0.9	5.0 \pm 1.2	4.7 \pm 1.1
Range	4 – 6	5 – 6	4 – 7	4 – 6
Water retention				
Mean \pm SD	5.8 \pm 1.7	6.6 \pm 1.6	5.5 \pm 1.2	5.6 \pm 1.4
Range	5 – 8	5 – 9	4 – 7	5 – 8
Negative affect				
Mean \pm SD	12.6 \pm 4.1	13.2 \pm 3.3	12.7 \pm 3.8	13.1 \pm 4.3
Range	8 – 17	10 – 17	9 – 16	8 – 17
Arousal				
Mean \pm SD	12.1 \pm 5.7	10.3 \pm 3.8	11.1 \pm 4.2	11.6 \pm 5.3
Range	6 – 19	7 – 16	6 – 16	6 – 18
Control				
Mean \pm SD	6.9 \pm 1.1	6.9 \pm 0.8	7.1 \pm 1.3	6.8 \pm 1.0
Range	6 – 8	6 – 8	6 – 9	6 – 8

Premenstrual phase – average of 7 days prior to onset of menses. Early follicular phase – day of menses onset to end of menses. Late follicular phase – after menses to EDO. Luteal phase – day after EDO to the day prior to onset of menses

For pain, the 1-way ANOVA revealed a significant effect of phase ($P = 0.001$). However, post hoc paired t-tests did not identify any specific phase differences (all $P > 0.05$). The average pain score appeared highest, however, in the early follicular phase (termed menses), with a large effect size (Cohen's $d = 0.88$) observed for the difference between means of premenstrual and early follicular phases. See table 6.

Analysis revealed no significant difference for concentration ($P = 0.436$), behavioural change ($P = 0.320$), autonomic reactions ($P = 0.052$), water retention ($P = 0.221$), negative affect ($P = 0.337$), arousal ($P = 0.187$) and control ($P = 0.590$) across the menstrual phases.

Brums & menses, day of 1st positive LH and 7 days after 1st positive LH

There was no significant difference across the menstrual cycle for anger ($P = 0.150$), confusion ($P = 0.160$), depression ($P = 0.223$), fatigue ($P = 0.368$), tension ($P = 0.497$) and vigour ($P = 0.811$). See table 7.

Readiness to invest physical and mental effort during menses, day of 1st positive LH and 7 days after 1st positive LH

The 1-way ANOVA revealed no significant difference across the menstrual cycle for readiness to invest physical ($P = 0.645$) and mental effort ($P = 0.133$). See table 8.

Table 7 BRUMS across menses, 1st positive LH and 7 days after 1st positive LH

Menstrual phase			
Subscale	Menses (days 2 – 4)	1 st Positive LH	7 days after 1 st positive LH
Anger			
Mean \pm SD	2.1 \pm 1.6	0.5 \pm 0.5	1 \pm 1.2
Range	1 – 4	0 – 1	0 – 2
Confusion			
Mean \pm SD	2.3 \pm 1.9	1.8 \pm 1.5	1.4 \pm 1.7
Range	0 – 5	1 – 4	0 – 3
Depression			
Mean \pm SD	2.3 \pm 1.2	0.5 \pm 0.9	0.7 \pm 0.6
Range	0 – 4	0 – 2	0 – 1
Fatigue			
Mean \pm SD	4.9 \pm 1.7	3.2 \pm 2.0	1.9 \pm 2.4
Range	3 – 7	1 – 5	1 – 5
Tension			
Mean \pm SD	4.4 \pm 3.1	3.7 \pm 3.2	3.3 \pm 3.7
Range	1 – 6	0 – 6	0 – 7
Vigour			
Mean \pm SD	2.6 \pm 0.7	3.8 \pm 3.4	4.5 \pm 4.0
Range	1 – 4	0 – 7	2 – 9

Table 8 Readiness to invest physical and mental effort during menses, day of 1st positive LH and 7 days after 1st positive LH

Menstrual phase			
Subscale	Menses (days 2 – 4)	1 st Positive LH	7 days after 1 st positive LH
Physical Effort			
Mean \pm SD	63.1 \pm 17.3	67.0 \pm 21.2	75.2 \pm 4.5
Range	40 – 77	46 – 86	71 – 79
Mental Effort			
Mean \pm SD	67.6 \pm 14.6	78.2 \pm 8.5	83.5 \pm 13.0
Range	54 – 76	71 – 83	74 – 93

Oral contraceptive user results

BBT and consumptions vs. withdrawal phase

A paired samples t-test revealed no significant difference between BBT in the consumption and withdrawal phase ($P = 0.388$), with the average temperature $36.20 \pm 0.28^{\circ}\text{C}$ and $36.25 \pm 0.25^{\circ}\text{C}$ in the consumption and withdrawal phase, respectively, a difference of 0.05°C .

Consumption phase was defined as the 21 days of active pill consumption and withdrawal phase was defined as the 7 days of placebo/no pill consumption

MDQ and start of withdrawal phase vs. end of withdrawal phase

Analysis revealed no significant difference for pain ($P = 0.564$), concentration ($P = 0.695$), autonomic reactions ($P = 0.317$), water retention ($P = 0.076$), negative affect ($P = 0.907$), arousal ($P = 0.224$) and control ($P = 0.083$). See table 9.

A paired samples t-test revealed a significant difference between the start and end of withdrawal phase ($P = 0.001$) for behavioural change. Behavioural change was higher at the start of the withdrawal phase compared to the end of withdrawal phase (6.8 ± 2.0 vs. 6.3 ± 2.0 , respectively). The behavioural subscale in the MDQ is comprised of symptoms including lowered school or work performance, take naps/stay in bed, stay at home, avoid social activities and decreased efficiency.

Table 9. MDQ and start and end withdrawal phase. * $P < 0.05$.

	Withdrawal phase	
Subscale	Start of phase	End of phase
Pain		
Mean \pm SD	11.5 \pm 4.6	11.4 \pm 4.2
Range	8 – 17	8 – 17
Concentration		
Mean \pm SD	11.0 \pm 2.5	10.7 \pm 2.5
Range	8 – 14	9 – 14
Behavioural change *		
Mean \pm SD	6.8 \pm 2.0	6.3 \pm 2.0
Range	5 – 10	5 – 10
Autonomic reactions		
Mean \pm SD	4.6 \pm 0.5	4.8 \pm 0.7
Range	4 – 5	4 – 6
Water retention		
Mean \pm SD	6.4 \pm 3.0	5.4 \pm 2.1
Range	4 – 12	4 – 9
Negative affect		
Mean \pm SD	13.4 \pm 5.2	13.3 \pm 6.6
Range	9 – 21	8 – 24
Arousal		
Mean \pm SD	8.9 \pm 3.1	8.1 \pm 2.9
Range	5 – 13	5 – 13
Control		
Mean \pm SD	7.2 \pm 1.7	6.8 \pm 1.2
Range	6 – 10	6 – 9

Start phase defined as days 22 – 25. End phase defined as days 26 – 28.

BRUMS and consumptions vs. withdrawal phase

Analysis revealed no significant difference between consumption phase and withdrawal phase for anger ($P = 0.770$), confusion ($P = 0.655$), depression ($P = 0.414$), fatigue ($P = 0.253$), tension ($P = 0.135$) and vigour ($P = 0.104$). See table 10.

Table 10 BRUMS across consumption and withdrawal phase

	Oral contraceptive phase	
Subscale	Consumption phase	Withdrawal phase
Anger		
Mean \pm SD	0.7 \pm 0.8	15 \pm 2.7
Range	0 – 2	0 – 7
Confusion		
Mean \pm SD	1.0 \pm 1.8	1.3 \pm 2.1
Range	0 – 5	0 – 5
Depression		
Mean \pm SD	0.5 \pm 0.9	1.5 \pm 2.1
Range	0 – 2	0 – 6
Fatigue		
Mean \pm SD	2.9 \pm 2.1	3.9 \pm 1.9
Range	0 – 6	2 – 7
Tension		
Mean \pm SD	1.9 \pm 1.9	2.8 \pm 2.5
Range	0 – 5	0 – 7
Vigour		
Mean \pm SD	5.0 \pm 2.7	3.3 \pm 0.5
Range	2 – 9	3 – 4

Readiness to invest physical and mental effort across consumptions and withdrawal phase

No significant difference was revealed for readiness to invest physical ($P = 0.257$) and mental effort ($P = 0.069$) between the consumption and withdrawal phase. See table 11.

Table 11 Readiness to invest physical and mental effort across consumption and withdrawal phase

	Oral contraceptive phase	
Subheading	Consumption phase	Withdrawal phase
Physical effort		
Mean \pm SD	72.1 \pm 14.7	72.1 \pm 15.6
Range	49 – 87.5	48 – 86.3
Mental effort		
Mean \pm SD	63.4 \pm 28.5	60.6 \pm 25.6
Range	16 – 91	13.5 – 87.7

V. Discussion

The purpose of this study was to determine if menstrual cycle phase or monophasic oral contraceptive use influenced body temperature, mood and somatic symptoms. We also aimed to investigate the utility of self-monitoring methods including self-report/calendar based, BBT and urine LH measurements. Participants logged and tracked their menstrual cycles over 2 – 3 months (or 2 – 3 complete menstrual cycles), using at home data collection methods including BBT, urinary luteinising hormone detection kits and self-report logs. Participants also completed several questionnaires throughout each cycle, including MDQ, BRUMS and readiness to invest physical and mental effort. The current study found that BBT, mood and somatic symptoms did not differ across the menstrual cycle. The similarity between the two distinct phases in the menstrual cycle found in the current study disagrees with the findings of others. In line with previous findings, we failed to find significant differences for the majority of parameters measured for OC users either between the OC consumption phase and OC withdrawal phase or between the start and end of the OC withdrawal phase. Although, interestingly, a significant difference was found in behavioural change between the start and end of withdrawal week in OC users.

Body temperature and eumenorrheic females

In the current study, we compared BBT between the follicular and luteal phase and the mid follicular and mid luteal phase, both comparisons showing no significant difference. This contrasts with findings of Dutton, Blanksby, and Morton (1989) who found that BBT increased significantly between the follicular phase and luteal phase, with a difference of 0.32°C. Similarly, Schoene, Robertson, and Pierson (1981) noted a significant difference between the mid follicular and mid luteal phase with a difference of 0.5°C. It should be noted, however, that participants in Schoene, Robertson, & Pierson's (1981) study, measured BBT at resting on test days compared to in the morning before getting out of bed, when temperatures lower. Refinetti (2010) demonstrated that body temperature increases throughout the day and is influenced by environmental and biological factors. Moreover, significant differences between the two main phases have been found by other researchers (Baker, Mitchell, et al., 2001; Baker, Waner, et al., 2001; Cagnacci et al., 2002; Shechter, Varin, & Boivin, 2010; Vidafar et al., 2018). Although it should be highlighted that a majority of the studies investigated core body temperature rather than BBT. Core body

temperature, compared to BBT, can be collected throughout the day/night to detect changes that occur at any time. Central thermometers, like those used when measuring core body temperature, have higher rates of accuracy and are considered the gold standard compared to peripheral thermometers, used when measuring BBT (Hernandez & Upadhye, 2016). BBT can be influenced by lifestyle factors such as stress, alcohol consumption, sleep, diet, nutritional factors, medications and illness (Barron & Fehring, 2005). Moreover, as mentioned previously, BBT can be challenging to interpret, with many studies relying on experienced researchers to study the graphs. Similarly, Gudgeon, Leader, & Howard (1990) found that only 40% of participants in their study demonstrated a shift within one day and 75% did within two days of the LH surge, and in 34% of individuals, the BBT charts differed by more than two days or were inconclusive. It is important to note that, caution should be taken when comparing results from studies that have used gold standard techniques and those that have not, as temperature readings may vary (Kelly, 2006; Rabinowitz, 1996).

The rise in body temperature during the second part of the menstrual cycle has been attributed to the rise in progesterone levels during the luteal phase (Grant et al., 2020; Isreal & Schneller, 1950; Stachenfeld et al., 2000). Whereas the decrease in temperature during the follicular phase has been attributed to oestrogens hypothermic effect. Cagnacci et al. (2002) and Grant et al. (2020) however found that the best predictor of body temperature changes is the progesterone/estradiol ratio. The absence of significant findings in the current study may be due to the way in which temperature was measured i.e., measuring BBT rather than core body temperature. The latter method would have been impractical and unfeasible for the current study, whereas BBT measurements could be instructed clearly and carried out at home easily and were therefore deemed to be the best way to collect data.

The current study also failed to find a significant difference in temperature across 8 time points surrounding the LH surge and EDO. As mentioned in the literature review, BBT has been seen to decrease, reaching its lowest point, approximately 1 day prior to the LH surge (Kawamori et al., 2019; Su et al., 2017; Tenan et al., 2016, 2013). The lack of significant findings in the current study may be due to the inaccuracy of the BBT, as mentioned above, and/or the LH testing methodology as discussed below.

Luteinizing hormone analysis

The mean LH spike in the current study occurred at 13.8 ± 2.1 days into the cycle, lasting 2.3 ± 1.2 days, with EDO occurring at 14.8 ± 2.1 days. Cole, Ladner, and Byrn (2009) measured the mean LH peak (the highest value in the peak period) which occurred at 14.7 ± 3.7 days, with a range of 10 – 20 days. Similar results were collected by Park, Goldsmith, Skurnick, Wojtczuk, and Weiss (2007) who found that the LH surge occurred on day 14.5 ± 3.6 (defined as the first LH value with a visually marked increase compared to prior value). As mentioned in the literature review, it is important to note that even if participants experienced an LH surge, it does not necessarily mean they had an ovulatory cycle (Krotz et al., 2005). It is therefore useful to look at other markers, such as serum LH, FSH and estradiol, progesterone and urinary pregnanediol 3-glucuronide (Ecochard et al., 2013; Li, Chen, Overstreet, Nakajima, & Lasley, 2002) as well as urinary LH to determine if individuals experienced ovulatory cycles.

In the current study, we asked participants to conduct LH tests at the same time each day (between the hours of 10:00-20:00, with the ideal standardised time of 10:30 am, to minimise potential confounding effects of diurnal variations). The guidelines were in line with the instructions on the LH at home test kits used (Urine test strip, 20mIU/mL 2.5mm, One Step, Home Health UK), which advised to avoid testing samples first thing in the morning, as LH would not yet appear in the urine due to synthesis occurring during the early morning. Krotz et al. (2005) investigated the time of day the LH surge occurs and found that most participants surged between 06:00-08:00 with lower surge rates occurring as the day goes on (08:00-19:00). Similar results were found by (Cahill, Wardle, Harlow, & Hull, 1998). It has been suggested that melatonin may be responsible for the timing of the LH surge. Melatonin is secreted from the pineal gland and is involved in sleep regulation and circadian rhythm amongst other things. Melatonin secretion increases during the evening, with darkness increasing production and light decreasing production, and peaks between 02:00 and 04:00 before gradually decreasing into the early morning. Melatonin has been seen to act on the gonads, reducing the secretion of gonadotrophins including LH (Reiter, 1980) and therefore, during the early morning when melatonin levels are decreasing, the LH surge occurs (Cahill et al., 1998; Grivas, Vasiliadis, Mouzakis, Mihos, & Koufopoulos, 2006). It is therefore plausible that participants who did not experience an LH surge, and were regarded as experiencing an anovulatory cycle, may have missed their surge (earlier in the day if testing

later) when testing, or that it would have been more likely to detect higher levels/pick up the surge with earlier sample times. However, whilst it is possible that LH levels are higher earlier in the day, it may not have been enough to cause missed/false negative readings if individuals took their samples later in the day. Nevertheless, timing of LH tests would be something to consider in future research, with LH tests carried out earlier in the day compared to later in the day.

To increase accuracy of testing, it would be advisable that participants carry out multiple LH tests through the day to aid in determining the most common time LH surges occur on an individual basis. However, this would increase the participant burden may also compromise study adherence. Furthermore, the LH test kits do not determine LH concentration, but rather only show positive vs negative based on a pre-determined threshold. This means that even if an individual receives a positive LH reading (i.e., sensitivity threshold is exceeded), it may not be the peak and therefore counting days using this method may prove inaccurate. A more accurate way would be to measure serum LH repeatedly throughout the day(s) (O'Connor et al., 2006), however this is an invasive technique and cannot be carried out by the participant themselves. Another repeated measure could be through collection of urine which can be analysed in a laboratory setting for LH concentration and/or other hormone assessments (Ecochard et al., 2013; Leiva, Bouchard, Abdullah, & Ecochard, 2018; H. Li et al., 2002). The above methods are however an extra burden to participants as multiple samples would need to be collected, stored and transported to a laboratory compared to using a LH stick which is able to give an instant result. It is worth noting that the majority of the studies mentioned are concerned with the fertility aspect of ovulation, but in the current study we used LH to confirm ovulatory cycles, to help distinguish between the two main phases.

Psychological effects and eumenorrheic females

Results of the repeated measures ANOVA indicated that 7 out of 8 symptom subscales in the MDQ did not differ significantly across the menstrual cycle phases (premenstrual, early follicular, late follicular and luteal phase). We did, however, discover a significant main effect of menstrual phase for pain ($P = 0.001$), although post hoc analysis did not identify any specific phase difference (all $P > 0.05$). The absence of significant difference between specific phases could be due to the sample size reducing the statistical power and chance of detecting a true effect (Button et al., 2013). However, there was a large effect size (Cohen's d

– 0.88) observed for the difference between means of premenstrual and early follicular phases, which would be in agreeance with previous literature. The pain subscale in the MDQ consists of 6 items (muscle stiffness, headache, cramps, backache, fatigue and general aches and pains) which are typically associated with menstruation. Mohamadirizi and Kordi (2013), through the use of the MDQ, discovered that females experienced pain throughout their menstrual cycle but particularly during bleeding, with 83.55% of females suffering during menses compared to 12.95% and 18.07% experiencing pain during pre-menstruation and post menstruation, respectively. Ross, Coleman, and Stojanovska (2003) found similar results. Their study found somatic symptoms, which included cramps, backache and general aches and pains, was higher during menses compared to the premenstrual phase. Comparable conclusions have been drawn by Boyle and Grant (1992), Heitkemper and Jarrett (1992), Lu (2001) and Van Den Akker and Steptoe (1985). However, we did not find any specific phase difference.

In contrast to previous studies, negative affect did not differ significantly between menstrual phases. Negative affect has 8 items in the MDQ, including crying, loneliness, anxiety, restlessness, irritability, mood swings, depression, and tension. Similarly, negative subscales in the BRUMS, including depression, tension and anger did not differ between menstrual phases. Many studies have suggested that scores for negative mood state were statistically higher in the late luteal phase (premenstrual phase) compared to menses (early follicular phase) and late follicular phase. Gonda et al. (2008) studied healthy eumenorrheic females, over 3 complete menstrual cycles, using the prospective record of the impact and severity of menstrual symptoms (PRISM), the study found that > 50% of participants had $\geq 66\%$ increase in physical symptom severity in the late luteal phase compared to the late follicular phase. Throughout the first menstrual cycle, participants completed a variety of questionnaires, including the state anxiety scale of the State Trait Anxiety Inventory (STAI), Symptom Distress Checklist 51 (SCL-51) and the Zung Self-rating depression scale at three time points: early follicular phase (3 – 4 days after onset of menstruation), the late follicular (8 – 10 days after onset of menstruation) and the late luteal phase (2 – 3 days preceding the onset of menses). The study found that scores for anxiety and depression were significantly higher in the late luteal phase compared to the follicular phase. Although, it should be highlighted that when the follicular phase was split into two subphases (early and late), SCL anxiety score were only significantly different when comparing the late follicular and late luteal phase. Whereas SCL depression scores were significantly different between early

follicular and late follicular phase and late follicular and late luteal phase. Moreover, Ziomkiewicz et al. (2012) found that 75% of females reported an increase in aggression, irritation and depressive behaviour during the luteal phase. Ross et al. (2003) found that negative affect symptomology peaked on day 27 in naturally cycling females (cycles were standardised into a 28-day record).

Mohamadirizi and Kordi (2013) conducted a retrospective questionnaire to analyse the association between menstruation and anxiety, depression and stress. The questionnaire results were split into three phases: menstrual signs a week before menstruation (luteal phase), during bleeding (menses) and a week after menstruation (follicular phase). The study found a positive correlation between the menstruation signs (including irritability, low back pain, fatigue, cramps) at all three time points and depression, anxiety and stress. Similar results were concluded by Nillni et al. (2013). Iqbal et al. (2021) found that 56.4% of women suffered with anxiety before or during menstruation, with 70.6% suffering with it before, 27.8% during and only 16% after, highlighting that the majority of women suffer with anxiety in the luteal phase compared to the follicular phase

In respect of psychological differences between participants, a number of studies have been carried out. Hardie (1997) conducted a study whereby females were blind to the menstrual cycle focus. Participants were instructed to complete a diary and questionnaires at the same time each day, either first thing in the morning or last thing in the evening. The diary entries/ratings reflected the previous 24 hours. The study used the counting method to determine participants menstrual cycle phase: premenstrual (7 days prior to onset of menses), menstrual (all days of bleeding), postmenstrual (7 days following menstruation) and intermenstrual (all remaining days). The study found that there was no recurrent pattern of premenstrual affective change over 2 cycles, even though 40% of participants perceived themselves to have PMS. Moreover, Balaha, Amr, Al Moghannum, and Al Muhaidab (2010) found that prevalence of anxiety and depression was significantly more evident in females who suffer with and are diagnosed with PMS, therefore to improve future research, studies should carry out preliminary testing on participants so that comparisons can be made fairly and accurately. Although, caution should be taken when analysing findings from studies that rely on the counting method to determine menstrual phases, as self-reports can be unreliable and lack in validity (Johnson, Miro, Barrett, & Ellis, 2009; Small, Manatunga, & Marcus, 2007; Wideman, Montgomery, Levine, Beynnon, & Shultz, 2013). Moreover, without

employing LH tests to confirm ovulatory cycles, anovulatory females could be included in the data.

It seems intuitive to assume that if females are experiencing somatic and affective symptoms during their menstrual cycle, particularly during the luteal and menses phase, this would have an effect on their readiness to exert physical and mental effort, whereas during the late follicular phase / ovulation, higher levels of effort/motivation may be expected (Cook et al., 2018). In the current study, however, we found no significant difference between menses, day 1 of positive LH test (late follicular/ovulation) and 7 days after positive test (mid luteal). It should be noted that readings for both physical and mental effort were higher, but not significant, 7 days after a positive LH test and lowest during menses. These findings are in contrast to the findings of Cook, Kilduff, and Crewther's (2018). They studied 22 athletes (elite and non-elite participants). Their participants were instructed to rate their motivation to train and compete on a Likert scale with 1 being no motivation train/compete and 7 being extremely motivated to train/compete. As would be expected, elite athletes had higher motivation to compete than the non-elite group. Moreover, the study found that both groups of athletes reported higher motivation to train on day 14 (ovulation) vs day 7 (follicular phase) and day 21 (luteal phase). It is important to highlight that the researchers only had participants self-report day 1 of their menstrual cycle (onset of menses) and then presumed day 14 would be ovulation day, however, as already mentioned, this methodology has its disadvantages. Li, Lloyd, and Graham (2020), however, investigated physical and mental fatigue across the menstrual cycle. They assessed individuals' physical and mental fatigue severity using the fatigue and energy scale and found that physical fatigue remained unchanged throughout the cycle when looking at three time points (10-14 days prior to ovulation and 5-10 days after ovulation). Whereas mental fatigue significantly increased from early follicular phase to mid luteal phase. The authors noted that mental fatigue may be higher during the mid-luteal phase due to the rise in both oestrogen and progesterone.

COVID-19 pandemic and the menstrual cycle

In December 2019, COVID-19 spread all over the world, with a global pandemic declared in March 2020. The COVID-19 pandemic has had a significant impact on the population's mental health (Salari et al., 2020). Periods of psychological distress and stress can affect a women's menstrual health. Stress activates the hypothalamic pituitary-adrenal axis within the

body (Smith & Vale, 2006; Toufexis, Rivarola, Lara, & Viau, 2014), once activated, there is an increase in cortisol and corticotropin-releasing hormone levels, which can suppress normal levels of reproductive hormones (Kalantaridou, Makrigiannakis, Zoumakis, & Chrousos, 2004), including LH, testosterone, oestrogen and progesterone. Phelan, Behan, and Owens (2021) found that as a result of the pandemic, women experienced reproductive health disturbances which were associated with a significant increase in suffering from mental health symptoms such as low mood, anxiety and poor concentration. The findings of Phelan, Behan, and Owens (2021) study are in agreeance with Demir, Sal and Comba (2021) in which anxiety scores were significantly higher during the pandemic compared to before the pandemic. Moreover, they found that females experienced an increase in PMS symptoms compared to pre-COVID, with 53% stating a worsening in symptoms, 7% stating an improvement in symptoms and 40% stating symptoms were unchanged. Similarly, Takmaz, Gundogmus, Okten, and Gunduz (2021) noted an increase in COVID-19 pandemic-induced anxiety, perceived stress and depressive symptoms. Comparable results were found by Bruinvels et al. (2021) when investigating eumenorrheic females and HC users.

It is interesting to note that differences in results of the current study and those mentioned within the literature review and discussion may be due to psychological differences due to the data collection during the pandemic. Without hormone analysis, or baseline measures (both physiological and psychological) during normal/non-Covid times, it is challenging to make assumptions, although, the pandemic may have influenced the participants reproductive hormones which may have played a role in the study's findings. For example, if testosterone levels were suppressed, this may explain why no significant findings were found between the two phases within the menstrual cycle when looking at readiness to invest effort. In line with this, Bruinvels et al. (2021) highlighted that 59.3% of participants stated they experienced a lack of motivation during the pandemic.

Body temperature and monophasic oral contraceptive users

Our finding that monophasic OC users have similar temperatures across the two phases (pill consumption and withdrawal) are in agreeance with results produced by Baker, Mitchell, and Driver (2001). However, in contrast, the OC pill consumption phase mean temperature was not similar to that of the non-oc luteal phase mean temperature (Baker, Mitchell, et al., 2001; Baker, Waner, et al., 2001). This may be due to the methodology differences, as both studies aforementioned used rectal temperatures over a 24 h period, whereas the current study

measured BBT in the morning using an oral thermometer. Discrepancies between the two methods and why it may have affected results can be read in the non-oc discussion section.

Psychological effects and monophasic oral contraceptive users

In the current study, we failed to find a significant difference between the start of the withdrawal phase and the end of the withdrawal phase in pain, concentration, autonomic reaction, water retention, negative affect, arousal and control. The findings are of interest as it demonstrated that during the first 5 days of withdrawal compared to the final 3 days of withdrawal no differences were found. Moreover, as the withdrawal bleed would have occurred during the withdrawal phase, starting in the first few days, it is interesting to note that individuals did not suffer from menstrual related symptoms that may have occurred. These findings are consistent with Sanders, Graham, Bass, and Bancroft (2001) who demonstrated that when compared to baseline measures (without the pill), oral contraceptives were perceived to reduce menstrual pain and other effects of menstrual related symptoms (Yonkers et al., 2017). Similarly, the concept that the oral contraceptives help stabilise the hormonal levels can be seen through the absence of significant differences between the consumption and the withdrawal phase for items of the BRUMS, including anger, confusion, depression, fatigue, tension and vigour, and in both subscales in the readiness to invest effort. In contrast, other researches have suggested that cyclic changes do occur throughout the cycle, particularly during the premenstrual and menses phase (Kelly et al., 2010; Walker & Bancroft, 1990), in OC users, although the changes are smaller when compared to non-oc users (Taggart et al., 2018; Yonkers et al., 2017). Hamstra, de Kloet, de Rover, and Van der Does (2017) found that during the pill withdrawal week, OC users reported increased irritability (Coffee, Kuehl, Willis, & Sulak, 2006). The increase in irritability may be due to the decrease in stabilising hormones, i.e., reduction in exogenous hormones, as recordings were completing on day 6 of the withdrawal week (Coffee et al., 2007; Kelly et al., 2010). The symptoms may increase during the withdrawal week due to exogenous hormonal concentrations decreasing and symptoms related to bleeding (Sulak et al., 2002). As mentioned in the literature review, the dose/type of progestin used may have an influence on whether females experience PMS symptoms / the severity of PMS experienced, Schultz-Zehden and Boschitsch (2006) recommended using a combined oral contraceptive containing EE 20 µg and drospirenone 3mg to help reduce/decrease premenstrual symptoms. Similarly, other low dose monophasic OC have been shown to reduce/decrease premenstrual symptoms,

such as desorgestrel, gestodene and norgestimate (London, 1992; Wichianpitaya & Taneepanichskul, 2013). In the current study, five out of six participants were prescribed one of the above Ocs. It is possible, therefore, that the absence of cyclic changes in the current study are due to the dose of OC used. It would be of interest for future studies to look at other OC doses. Moreover, the variety of dose's used in the current study highlight a limitation within the paper. It would have been more beneficial to recruit females who used the same type, brand and dose of OC for fair comparisons to be made with other studies.

In contrast, a significant difference was seen in behavioural change, with changes greater at the start of the withdrawal phase compared to the end. Behavioural change includes lowered school or work performance, take naps/stay in bed, stay at home, avoid social activities and decreased efficiency. The start of the withdrawal phase would comprise the reduction and gradual decrease of exogenous hormones and a gradual increase of endogenous estradiol, although it should be noted that the change in endogenous hormones are not significantly different between the two phases (consumption and withdrawal) (Baker, Mitchell, & Driver, 2001). Moreover, the reduction of the exogenous hormones signals the start of the premenstrual and menses phase (withdrawal bleed) within the OC users. It would therefore be probable to assume that compared to the later end of the withdrawal phase, the women experienced symptoms such as a reduction in energy which would have influenced their behaviour i.e., staying in bed, taking naps and decreased efficiency. Although, it should be highlighted that when fatigue was examined through the BRUMS questionnaire, comparing consumption and withdrawal, there was no significant difference found. The inconsistencies within the results could be due to the fact that behavioural changes were only evident for the first half of the withdrawal period and were reduced dramatically towards the latter, which would therefore aid in highlighting why no significant difference was found in the BRUMS (recorded on days 5 – 7 in withdrawal phase). Furthermore, premenstrual symptoms tend to peak ~2 days prior to the onset of menses (Yonkers et al., 2008). In the current study, OC users had a mean menses start date of the 4th day in the withdrawal phase, therefore if symptoms were to occur and peak, it would be captured at the start of the withdrawal phase compared to the end.

Limitations

In interpreting the results of the current study, several limitations must be taken into account. The assessments made were through analysis of questionnaires and relied on self-report. Self-report questionnaires can have great variability, as individuals may not know how to fill them in correctly and/or the individuals may be dishonest and give incorrect details. Moreover, due to the at home setting, we were not able to conduct serum hormone concentrations to validate the menstrual phase.

As mentioned within the discussion, BBT recordings can be influenced by a variety of things (Barron & Fehring, 2005) that were not controlled in the current study. Furthermore, oral thermometers may not be as sensitive to temperature changes compared to central thermometers; central thermometers, have higher rates of accuracy and are considered the gold standard (Hernandez & Upadhye, 2016). Asadian, Khatony, Moradi, Abdi, and Rezaei (2016) demonstrated that when compared to nasopharyngeal temperature (inserted through the nasal cavity), the oral thermometer method had ‘acceptable precision’ in measuring body temperature although accuracy was lower. It should be highlighted that the aforementioned study is focused on the use of oral thermometers in a clinical setting, which was not the focus in the current study. BBT recording is useful, as patterns can be highlighted over several months of tracking, however it is advisable that BBT is used in conjunction with other tracking methods, such as urine LH which is a more appropriate method for identifying ovulation.

The current study controlled the type of oral contraceptive, however we failed to control the brand and dose, which may have caused discrepancies within the data, even more so when comparing our findings to others. Moreover, due to time restraints, the current study was unable to compare findings between non-oc users and monophasic OC users. It would therefore be important for future studies to investigate if differences occurred between the groups.

The sample size for both groups were low, which was unfortunate as it may have reduced the chance of detecting a true effect (Button et al., 2013). However, it was of utmost importance to investigate the differences of each group separately, to avoid placing them into the same

category, as discussed in the literature review, it is appropriate due to the differences in hormonal profile.

As previously discussed, this study has been limited by the COVID-19 pandemic. The study design was affected, and data collection was limited to at home-based recordings compared to laboratory based assessments, such as hormone analysis. Moreover, the psychological differences due to the pandemic may have influenced the results, which is challenging in two respects; the results collected may not represent the true cyclic changes within the menstrual cycle and the outcomes found in the study may be difficult to compare to previous and future studies. On the other hand, this study is unique as it conducted research during a global pandemic and was able to test the utility of at home tracking methodologies, which can be used for future studies.

Conclusions

The purpose of this study was to determine if menstrual cycle phase or monophasic oral contraceptive use influenced body temperature, mood and somatic symptoms. We also aimed to investigate the utility of self-monitoring methods such as self-report/calendar based, BBT and urine LH measurements. We found that BBT, mood and somatic symptoms did not differ across the menstrual cycle, which disagrees with the findings of others. We also failed to find significant differences for the majority of parameters measured for OC users either between the OC consumption phase and OC withdrawal phase or between the start and end of the OC withdrawal phase, with the exception a significant difference found in behavioural change between the start and end of withdrawal week. Moreover, the at home LH detection kits were effective, as participants were able to easily carry out and track their results. Although BBT recordings were simple to record, they were not effective in displaying temperature changes throughout the menstrual cycle in eumenorrheic females.

It would be useful for future research to conduct a similar study, on a larger population, to determine whether there are differences across the menstrual cycle in eumenorrheic females and in monophasic oral contraceptive users. Moreover, the validity and accuracy of oral thermometers used to record BBT should be investigated. Oral thermometers are an inexpensive and easy to use tool that many individuals already use to track their cycle, either for their own interest, to avoid pregnancy or to plan pregnancy in an at home environment,

therefore it would be a beneficial research area to investigate for the wider population. Further research is required to determine if changes occur across the menstrual cycle, and if changes do occur then how may the fluctuations influence sporting performance. Finally, something to consider for future research would be the impact the global pandemic has had on the menstrual cycle and how it may influence individual's psychology.

Practical applications

- Mood and somatic changes did not significantly differ across the menstrual cycle in naturally cycling women, these findings could further investigations into why some women may perceive changes rather than experience changes across the menstrual cycle.
- Monophasic oral contraceptive users overall did not experience significant changes across their menstrual cycle (consumption and withdrawal phase), which may reiterate the point that the synthetic hormones provide a controlled and steady environment

VI. References

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VII. Appendices

a. Original study overview

Inclusion criteria:

Healthy, active females aged 18 – 55 years old, and males, aged 18-45 years old. The participants should be active and train at a vigorous intensity for 75 minutes or at a moderate intensity for 150 minutes per week (UK Chief Medical Officers, 2019). During testing, participants should maintain their normal weekly training schedule (and maintain at the same level, in terms of frequency, intensity, duration etc, for the duration of the study). Female participants should be experiencing normal menstrual cycles (free from oral contraceptives for at least 3 months) or using a monophasic oral contraceptive for at least 3 months

Exclusion criteria:

Cardiovascular, respiratory and neuromuscular disease and mental impairment; injury in the last three weeks and upper respiratory tract infection in the last 2 weeks.

Pre-screening pilot and main study:

A pre-screening questionnaire (PAR-Q) will be completed. Anthropometric measurements (height and body mass) and baseline heart rate (HR) will be recorded via a HR monitor located around the chest (BHIP 9 HR monitor, UK). If meetings are required, they will be conducted either on the SSES premises, or if more convenient for participants, in a mutually convenient public location with reference to the relevant risk assessments (SSESRA50: Off-site testing at Sports clubs and facilities; SSESRA62: Out of hours working).

Pilot study methodology

The experimental design will be a single-blind study, as the participant will not know the % of peak power output. Randomisation of order will be completed by a computer generator.

Familiarisation

The first visit to Medway Park will be to familiarise the individuals with the protocol and equipment used during the trials. Pre-screening and anthropometric measurements, as mentioned above, will be completed.

The participants will complete a standardised 5-minute warm up on the cycle ergometer (Excalibur Sport, Lode, Groningen, The Netherlands) at a rating of perceived exertion of 11 (RPE – Borg scale 6 -20) (Borg & Löllgen, 2001). A 5 second maximal sprint at 3 minutes will also be performed. Participant's intensity (work rate) and cadence (rpm) at 11 RPE will then be used for subsequent warmups. After 5 minutes of rest, participants will then be instructed to complete the RS protocol; 10 x 6 s maximal sprints, interspersed with 30 s active no load recovery. Each sprint will be performed in a seated position with their feet secured to pedals, either using toe straps on pedals, or cleats/cycling shoes if they have their own. All participants will be verbally encouraged during each sprint to give maximal effort. After 20 minutes of recovery an interval-based TTE protocol, 30 s exercise, 30 s active no load recovery, will be completed at a fixed intensity: 25%, 30% 35% and 40% of peak power output (taken as an average of the first 2 RSA sprints).

Criteria to terminate test:

Participants will continue the interval protocol until volitional exhaustion (until they cannot continue any longer). Participants will need to keep at the target cadence during the fixed % of their PPO; if their cadence drops by > 10 rpm they will be given a warning and instructed to increase cadence back to the target otherwise the test will be ended. If they are able to increase, they may continue with a warning but if cadence drops > 10 rpm again the test will be ended (i.e., if they drop by > 10 rpm twice in the same interval the test will be terminated). The exact time of failure/withdrawal will be recorded (to the nearest second).

Once all testing has been completed, participants will complete a 5-minute cool down.

Experimental trials

Testing will be conducted at a time convenient for the laboratory, researchers and participants. The same time of day will be maintained for each participant throughout the trials.

As described above, the participants will perform a standardised warm up. A 5-minute rest will be given before the trial start. Each trial will consist of 10 x 6 s maximal sprints, interspersed with 30 s active no load recovery and an interval-based TTE protocol, 30 s exercise, 30 s active no load recovery, will be completed at a fixed intensity.

Standardisation of procedures

You will be required to complete a food and activity diary for the 48 hours before the first experimental visit. You will be asked to repeat your food and fluid intake (and activity) as closely as possible in the 48 hours prior to future experimental visits.

You will also be asked to refrain from caffeine-containing sources, alcohol and vigorous activity 24-hours prior to the trials. You will also be required to avoid food or beverages (except plain water) 3 hours prior to testing.

Main study methodology:

Methodology:

The experimental design will be a counterbalance, randomised, double-blinded design. Females in the study will complete 6 visits to Medway Park, 2 familiarisation and 4 main trials and males will complete 5 visits to Medway Park, 1 familiarisation and 4 main trials (see figure 6). After female participants have expressed interest in the study, a meeting will be arranged to discuss tracking of the menstrual cycle (booklet logging and BBT measures) and also fill in the menstrual history questionnaire. Meetings will be conducted either on the SSES premises, or if more convenient for participants, in a mutually convenient public location with reference to the relevant risk assessments (SSESRA50: Off-site testing at Sports clubs and facilities; SSESRA62: Out of hours working).

Familiarisation

The first visit to Medway Park will be to familiarise the individuals with the protocol and equipment used during the trials. Participants will be given a caffeine consumption booklet (modified Landrums (1992) prospective questionnaire) in which they will need to complete for a 7-day period. Full instructions will be given to participants.

A pre-screening questionnaire (PAR-Q) will be completed. Participants will then complete the following forms: motivation questionnaires, Brunel mood scale (BRUMS) and for females exclusively the menstrual distress questionnaire (MDQ) form T (Moos, 1968).

Anthropometric measurements (height and body mass) and baseline heart rate (HR) will be recorded via a HR monitor located around the chest (BHIP 9 HR monitor, UK). Saliva samples will be collected and processed (e.g., rendering acellular) for storage and later analysis of genotype and salivary immune markers.

The participants will then complete a standardised 5-minute warm up on the cycle ergometer (Excalibur Sport, Lode, Groningen, The Netherlands) at a rating of perceived exertion of 11(RPE – Borg scale 6 -20) (Borg & Löllgen, 2001). Participant's intensity (work rate) and cadence (rpm) at 11 RPE will then be used for subsequent warmups. Participants will then have a 5-minute rest before RS protocol. Participants will be instructed to complete the RS protocol; 10 x 6 s maximal sprints, interspersed with 30 s active no load recovery. Each sprint will be performed in a seated position with their feet secured to pedals, either using toe straps on pedals, or cleats/cycling shoes if they have their own. All participants will be verbally encouraged during each sprint to give maximal effort. After 10 minutes of recovery an interval-based TTE protocol, 30 s exercise, 30 s active no load recovery, will be completed at a fixed intensity (35% of individuals PPO, with cadence equal to the preferred cadence determined in warm-up) (see figure 6). The PPO will be determined by taking the average of the first 2 sprints in the familiarisation RS performance.

Criteria to terminate test:

Participants will continue the interval protocol until volitional exhaustion (until they cannot continue any longer). Participants will need to keep at the target cadence during the intervals at 35% of their PPO; if their cadence drops by > 10 rpm they will be given a warning and instructed to increase cadence back to the target otherwise the test will be ended. If they are able to increase, they may continue with a warning but if cadence drops > 10 rpm again the test will be ended (i.e., if they drop by > 10 rpm twice in the same interval the test will be terminated). The exact time of failure/withdrawal will be recorded (to the nearest second).

Once all testing has been completed, participants will complete a 5-minute cool down.

Experimental trials

Testing will be conducted at a time convenient for the laboratory, researchers and participants. The same time of day will be maintained for each participant throughout the trials. On arrival at the laboratory participants will be required to rinse their mouth with plain

water, then sit restfully for 10 min. Resting HR will be measured in the final 2 min and then saliva and blood samples collected. Next (approx. 1-hour prior to testing), participants will ingest a capsule, containing either 6 mg·kg⁻¹ body mass caffeine or placebo (microcrystalline cellulose). Participants will then complete the following forms: motivation questionnaires, BRUMS and for females exclusively the MDQ form T (Moos, 1968).

40 - 45 minutes post capsule ingestion baseline HR will be recorded.

As described above, the participants will perform a standardised warm up. A 5-minute rest will be given before the trial start. Each trial will consist of 10 x 6 s maximal sprints, interspersed with 30 s active no load recovery and an interval-based TTE protocol, 30 s exercise, 30 s active no load recovery, will be completed at a fixed intensity (35% of individuals PPO).

Immediately after the standardised cool down a blood sample will be taken (5 minutes post TTE). 9 minutes post TTE, participants will have 1 minute to drink / rinse their mouth with plain water, then sit restfully for 10 minutes. After 10 minutes, a saliva sample will be taken (20 minutes post TTE) (see figure 7).

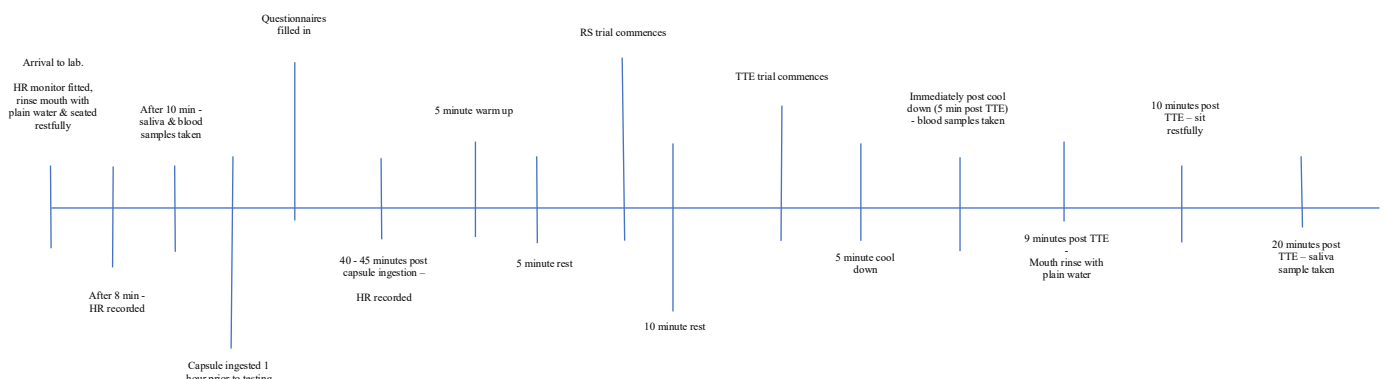


Figure 6 Schematic of original study - trial testing procedures

Study design

The study will be a crossover design, whereby each participant will service as their own control (Betts & Gonzalez, 2018) by ingesting a capsule containing either 6 mg·kg⁻¹ body mass caffeine or placebo (microcrystalline cellulose). The placebo capsule will be similar in shape, size and tastes to the caffeine capsule. The study will be double-blind, as the participant and researcher will not know what each capsule contains, until after the study has

been completed (to avoid any unconscious bias or expectancy effects). A manipulation check will be implanted after each experimental trial.

Randomisation of order will be completed by a computer generator . For females, during each cycle they will have a caffeine and placebo trial – the order of the substances within each phase of the menstrual cycle will be randomised. For males, it will be completely randomised with 2 trials of caffeine and placebo in any order (see table 2). Trials would take place in follicular (3 – 7 days post onset of menses) and luteal phase (20- 26 days post onset of menses) for naturally cycling females and consumption (7 – 21 days of OC consumption) and withdrawal phase (26 – 28 days in cycle) for oc users.

Table 12 Original study - randomisation of order throughout study

Female randomisation across trials			
Menstrual cycle 1		Menstrual cycle 2	
Trial 1	Trial 2	Trial 3	Trial 4
Caffeine	Placebo	Caffeine	Placebo
Placebo	Caffeine	Placebo	Caffeine
Male randomisation across trials			
Trial 1	Trial 2	Trial 3	Trial 4
Caffeine	Caffeine	Placebo	Placebo
Placebo	Placebo	Caffeine	Caffeine
Caffeine	Placebo	Caffeine	Placebo
Placebo	Caffeine	Placebo	Caffeine

Standardisation of procedures

You will be required to complete a food and activity diary for the 48 hours before the first experimental visit. You will be asked to repeat your food and fluid intake (and activity) as closely as possible in the 48 hours prior to future experimental visits.

You will also be asked to refrain from caffeine-containing sources, alcohol and vigorous activity 24-hours prior to the trials. You will also be required to avoid food or beverages (except plain water) 3 hours prior to testing.

b. Menstrual history questionnaire

1. How long is your menstrual cycle on average? How many days from your first menstrual period to the next menstrual period? The length of the menstrual cycle is the numbers of days between the first day of menstrual bleeding of one cycle to the day before the onset of menses of the next cycle.

-
2. How long are your periods? Day 1 of menstrual bleeding is when feminine protection (i.e., sanitary towels etc.) is required. The end of the period is defined as the last day in which feminine protection is required.

3. Do you take any oral contraceptives?

- a. Yes

If so, please name: _____

- b. No

4. Do you have irregular periods?

- a. Yes

- b. No

5. How often have you had your menstrual periods in the last year?

- a. Once every 20 days or less

- b. Every 21 – 27 days

- c. Every 28 – 35 days

- d. Every 36 – 50 days

- e. 51 days or more

6. How many of the symptoms below do you experience during your menstrual cycle.

Please tick all that are appropriate.

Insomnia	Confusion	Anxiety	Hot flashes	Distractible	Swelling (e.g., abdomen, breasts or ankles)
Crying	Headache	Backache	Nausea or vomiting	Depression	Change in eating habits
Muscle stiffness	Cramps	Cold sweats	Restlessness	General aches and pains	Bursts of energy or activity
Forgetfulness	Dizziness or faintness	Fatigue	Irritability	Mood swings	Lowered motor co-ordination

c. BRUMS questionnaire

Below is a list of words that describe feelings. Please read each one carefully. Then place a cross in the box that describes **HOW YOU FEEL RIGHT NOW**. Make sure you answer every question.

Date:

		Not at all	A little	Moderately	Quite a bit	Extremely
1	Panicky					
2	Lively					
3	Confused					
4	Worn out					
5	Depressed					
6	Downhearted					
7	Annoyed					
8	Exhausted					
9	Mixed-up					
10	Sleepy					
11	Bitter					
12	Unhappy					
13	Anxious					
14	Worried					
15	Energetic					
16	Miserable					
17	Muddled					
18	Nervous					
19	Angry					
20	Active					

21	Tired					
22	Bad Tempered					
23	Alert					
24	Uncertain					

d. Motivation questionnaire

Date:

Readiness to invest physical effort

Please mark the line below with a single downward stroke to indicate how physically ready you are to invest effort in this task.

Not ready at all _____ Complete readiness (ready give maximal effort)

Readiness to invest mental effort

Please mark the line below with a single downward stroke to indicate how mentally ready you are to invest effort in this task.

Not ready at all _____ Complete readiness (ready give maximal effort)

e. Menstrual distress questionnaire

For each symptom choose the descriptive category listed below which best describes your experience of that symptom **today**. Circle the number of the category which best described your experience of the symptom **today**.

	Descriptive categories	No reaction at all	Barely noticeable	Present, mild	Present, moderate	Present, strong	Acute or partially disabling
1	Weight gain	1	2	3	4	5	6
2	Insomnia	1	2	3	4	5	6
3	Crying	1	2	3	4	5	6
4	Lowered school or work performance	1	2	3	4	5	6
5	Muscle stiffness	1	2	3	4	5	6
6	Forgetfulness	1	2	3	4	5	6
7	Confusion	1	2	3	4	5	6
8	Take naps or stay in bed	1	2	3	4	5	6
9	Headache	1	2	3	4	5	6
10	Skin disorders	1	2	3	4	5	6
11	Loneliness	1	2	3	4	5	6
12	Feelings of suffocation	1	2	3	4	5	6
13	Affectionate	1	2	3	4	5	6
14	Orderliness	1	2	3	4	5	6
15	Stay at home from work or school	1	2	3	4	5	6
16	Cramps (uterine or pelvic)	1	2	3	4	5	6
17	Dizziness or faintness	1	2	3	4	5	6
18	Excitement	1	2	3	4	5	6
19	Chest pains	1	2	3	4	5	6
20	Avoid social activities	1	2	3	4	5	6
21	Anxiety	1	2	3	4	5	6
22	Backache	1	2	3	4	5	6
23	Cold sweats	1	2	3	4	5	6
24	Lowered judgment	1	2	3	4	5	6
25	Fatigue	1	2	3	4	5	6
26	Nausea or vomiting	1	2	3	4	5	6
27	Restlessness	1	2	3	4	5	6
28	Hot flashes	1	2	3	4	5	6
29	Difficult in concentration	1	2	3	4	5	6

30	Painful or tender breasts	1	2	3	4	5	6
31	Feelings of well-being	1	2	3	4	5	6
32	Buzzing or ringing in ears	1	2	3	4	5	6
33	Distractible	1	2	3	4	5	6
34	Swelling (e.g., abdomen, breast, ankle)	1	2	3	4	5	6
35	Accidents (e.g., cut finger, break dish)	1	2	3	4	5	6
36	Irritability	1	2	3	4	5	6
37	General aches and pains	1	2	3	4	5	6
38	Mood swings	1	2	3	4	5	6
39	Heart pounding	1	2	3	4	5	6
40	Depression (feeling sad or blue)	1	2	3	4	5	6
41	Decreased efficiency	1	2	3	4	5	6
42	Lowered motor coordination	1	2	3	4	5	6
43	Numbness or tingling in hands or feet	1	2	3	4	5	6
44	Change in eating habits	1	2	3	4	5	6
45	Tension	1	2	3	4	5	6
46	Blind spots or fuzzy vision	1	2	3	4	5	6
47	Bursts of energy or activity	1	2	3	4	5	6

f. Urine dipstick instructions

SPECIMEN COLLECTION:

We will ask you to start at some point between day 7 and 10 of your cycle (we will decide the specific day after we have gathered some preliminary information and discussed this with you). Once we identified what day you should begin testing you should then collect your urine on a daily basis for 10 consecutive days.

Important points

1. Do not use first morning urine samples as LH is synthesized in your body early in the morning. It will not show up in your urine until later in the day.
2. The best time to collect your urine is between 10 am – 8 pm. Pick a regular time that suits you best. Ideally, for this study try to do this at 10:30 am each day (if this fits with the requirement below, to reduce fluid intake for around 2 h before the sample).

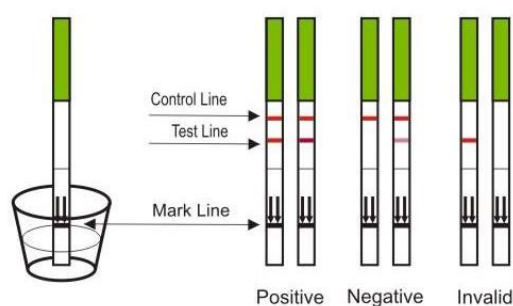
3. Collect urine at about the same time each day. Reduce liquid intake about 2 hours before collecting your urine as a diluted urine sample can prevent the test from detecting LH surge.

BEFORE YOU BEGIN

1. Read the instructions thoroughly before you begin.
2. Do not open the foil pouch until you are ready to begin the test.
3. Make sure you have a watch, clock or timer ready.
4. Make sure that the stick has been stored at room temperature (i.e., avoid keeping them in particularly hot areas, such as near heating or cooking appliances).

TEST PROCEDURE:

1. Determine the day to begin testing.
2. Collect urine sample in a clean and dry container.
3. To begin testing, open the sealed pouch and remove the strip. Do not remove the strip until you are ready to begin testing.
4. With the arrows pointing downwards towards the urine, place the test strip vertically (straight) into the urine sample, for at least 10 seconds. **DO NOT** allow the urine to go above the MARK level line.
5. Remove the strip from the urine and place on a clean, dry surface.
6. Wait for coloured bands to appear. Depending on the concentration of LH in the urine specimen, positive results may be observed within 1 minute. However, to confirm negative results, the complete reaction time of 10 minutes is required. Results obtained after 30 minutes may be considered invalid.



INTERPRETATION AND RECORDING OF RESULTS:

After each test, you must decide if you are having a L.H. surge. Please add the result to your daily log (record as either Positive or Negative). If the result is inconclusive, please repeat 1 further time.

To determine your result, you must compare the colour intensity of the test band to the control band. The control band is used to compare the test band against and also confirms that you have completed the test correctly.

Positive for L.H. surge

If two colour bands are visible and the test band **is of almost equal or greater colour intensity (darker)** than the control band, this is a positive result and a good indication that the L.H. surge is occurring.

Negative for L.H. surge

If two bands are visible but the test band is of a less intense colour (paler) than the control band or cannot be seen, this means the L.H. level is at or near its normal level and that the surge is not in progress. You should continue with daily testing.

Invalid result

If no control band appears within 5 minutes, the result is invalid and should be ignored. A visible control line is needed in all cases to confirm a proper test result. Repeat test with a new test kit.

g. Log booklet non-oc users

Daily logging booklet

For every day in the monitoring months complete:

- Menstrual day tracker
- Log your basal body temperature
- Physical activity level

Urine dip-stick:

- **Cycle1:** Please start this from day ____ and continue daily for 10 days
- **Cycle 2:** Please start this from day ____ and continue daily for 10 days

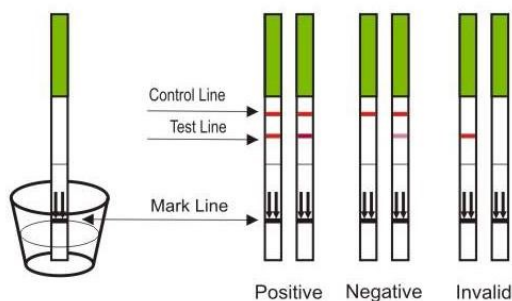
- **Cycle 3:** Please start this from day _____ and continue daily for 10 days

Instructions for basal body temperature:

- Record the temperature every morning before getting out of bed without sitting up.
- Ensure you take the reading at the same time every day
- Follow the directions for your thermometer to get the best results
- Record your temperature in the logbook

Instructions for urine dip-stick:

- Collect urine sample in a clean and dry container.
- To begin testing, open the sealed pouch and remove the strip. Do not remove the strip until you are ready to begin testing.
- With the arrows pointing downwards towards the urine, place the test strip vertically (straight) into the urine sample, for at least 10 seconds. DO NOT allow the urine to go above the MARK level line.
- Remove the strip from the urine and place on a clean, dry surface.
- Wait for coloured bands to appear. Depending on the concentration of LH in the urine specimen, positive results may be observed within 1 minute. However, to confirm negative results, the complete reaction time of 10 minutes is required. Results obtained after 30 minutes may be considered invalid.
- Please see full information on instruction leaflet for detailed instructions, and make sure you are familiar with this before testing.



Tracking of menstrual cycle

For each month below, place a 'S' or 'F' on the date you start (S) and finish (F) your period

The first day of your period – determined by midday (spotting does not count).

- If your period starts before 12:00 midday, that is the day your period has started.
- If your period starts after 12:00 midday, the following day is classified as the first day of your period.

The last day of your period – determined by midday.

- If your period ends before 12:00 midday, that is the day your period has finished
- If your period ends after 12:00 midday, the following day is classified as the last day of your period

Please also indicate the urine dipstick results (positive or negative) for relevant days, in the lower row of each section (**see template 1**)

Summary:

- **S – started period** **Positive** = dipstick positive result (i.e., 2 lines)
- **F – finished period** **Negative** = dipstick negative result (i.e., 1 line, or 2nd line very light)

Tracking of physical activity:

Please provide brief details of your current weekly levels of physical activity (sport, physical fitness or conditioning activities), using the following classification for exertion level:

L = light (slightly breathless)

M = moderate (breathless)

V = vigorous (very breathless)

Template two: example of tracking physical activity

Day & Date:	Activity	Duration (mins)	Level
Monday 13/07	Spin	45	V
Tuesday 14/07			
Wednesday 15/07	Yoga	30	L
Thursday 16/07	5 km run	32 min	M
Friday 17/07			
Saturday 18/07			
Sunday 19/07			

Template one: example of completed tracking log

		Days in the month																														
	Month	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31
Period	July					S							F																			
Temp																																
Urine dipstick															Neg	Neg	Neg	Neg	Neg	Neg	Post	Pos	Neg									

		Days in the month																														
	Mont h	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31
Period																																
Temp																																
Urine dipstick																																
Period																																
Temp																																
Urine dipstick																																
Period																																
Temp																																
Urine dipstick																																

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- h. Log booklet oc users

Daily logging booklet

For every day in the monitoring months complete:

- Menstrual day tracker
- Log your basal body temperature
- Log your physical activity

Instructions for basal body temperature:

- Record the temperature every morning before getting out of bed without sitting up.
- Ensure you take the reading at the same time every day
- Follow the directions for your thermometer to get the best results
- Record your temperature in the logbook

Questionnaires:

- MDQ – print out
 - Complete during withdrawal phase (7 days)
- BRUMS & Motivation
 - Pill consumption phase:
 - day 8
 - day 9
 - day 10
 - Pill withdrawal phase:
 - day 5 / 26 (post pill)
 - day 6 / 27 (post pill)
 - day 7 / 28 (post pill)

Tracking of menstrual cycle

For each month below, place a 'S' or 'F' on the date you **start (S)** and **finish (F)** your period

The first day of your period – determined by midday (spotting does not count).

- If your period starts before 12:00 midday, that is the day your period has started.
- If your period starts after 12:00 midday, the following day is classified as the first day of your period.

The last day of your period – determined by midday.

- If your period ends before 12:00 midday, that is the day your period has finished
- If your period ends after 12:00 midday, the following day is classified as the last day of your period

Place a 'X' on the date you **stop** taking your oral contraceptive and a 'O' on the day you **start** taking your oral contraceptive.

Summary:

- S – started period
- F – finished period
- X – date stop taking oral contraceptive
- O – date you start taking oral contraceptive

Physical activity:

Please provide brief details of your current weekly levels of physical activity (sport, physical fitness or conditioning activities), using the following classification for exertion level:

L = light (slightly breathless)

M = moderate (breathless)

V = vigorous (very breathless)

Template two: example of tracking activity

Day & Date:	Activity	Duration (mins)	Level
Monday 13/07	Spin	45	V
Tuesday 14/07			
Wednesday 15/07	Yoga	30	L
Thursday 16/07	5 km run	32 min	M
Friday 17/07			
Saturday 18/07			
Sunday 19/07			

Template 1: Example of tracking oral contraceptive use, period & basal body temperature

		Days in the month																														
	Mont h	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31
Per iod & pill	July	x		S			F		O																							
		Temp	36.7	36.6	36.5																											

Menstrual tracking:

NB: temp is temperature

		ND: temp is temperature																															
		Days in the month																															
	Month	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	
Period & pill																																	
Temp																																	
Period & pill																																	
Temp																																	

Day & date	Activity	Duration (mins)	Level